The Botanical Review

Interpreting Botanical Progress

Founded and published by
H. A. GLEASON AND E. H. FULLING

Managed and edited at The New York Botanical Garden by
E. H. FULLING

Volume VII
1941

Published Monthly at
North Queen Street and McGovern Ave.
Lancaster, Pa.

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

THE BOTANICAL REVIEW

VOL. VII | JANUARY, 1941 | No. 1

HALOPHYTES

J. C. TH. UPHOF

Rollins College

INTRODUCTION

Halophytes are plants which obtain their food from a surrounding containing a higher percentage of salts than normal glykophytes are able to endure. By salt, NaCl is usually understood, but logically consideration should also be given those plants which are strongly influenced by other salts as well, *e.g.*, $MgSO_4$, $NaSO_4$, KCl.

Since halophytic plants themselves as well as their natural habitats are so distinctive, it is not surprising that they attracted the attention of the majority of writers on plants in past centuries. For example, Dodoens (73) mentions among halophytes in his Crüydeboeck, 1563, the occurrence of the Zee wechbree, *Plantago maritima,* in saline soils along dikes and margins of salt waters. Lobelius (192), in his Stirpium adversaria nova of 1570, says about *Salicornia:* "multo frequentissima ad quaslibet maritimus oras tum Oceani, tum Mediterranei maris. . . ." Blankaart (36), in De Nederlandsche Herbarius of Kruidboek, 1697, mentions Zee sterrekruid, *Aster Tripolium,* that grows in saline places near the Ij, but which could be successfuly grown in gardens near Amsterdam. Linnaeus, in his Hortus Cliffortianus, 1737, and Species Plantarum, 1753, frequently gives in his plant descriptions the saline localities of species. We read the same in some early descriptions of plants from the New World, as in the works of Sloane, 1696, 1707, Swartz, 1791, and others on *Batis maritima, Avicennia nitida, Annona uliginosa* and other salt plants. It is of historical interest to note that in those early days some works were published that were entirely devoted to marine plants, as the publications of Ludwig (193) in 1736, and of Valentijn (334) in 1754. Many also included a number of lower Metazoa.

The word halophyte is derived from halos (salt) and phuton

(plant); it was apparently introduced by Pallas, for Schrader (275, 276), mentioning a lecture given by the former in Göttingen in 1809, states that *Salicornia, Salsola* and related plants be put "unten einem wie er glaubt, passender Namen von Halophyta vereinigt."

The study of halophytes has frequently been a center of interest and is so at the present time. During the rise of our different divisions of botanical sciences, such as morphology, physiology, ecology and sociology, it has been regarded from these different angles, and at present much of the unknown is still left for exploration.

GENERAL ASPECTS

Halophytes are found among the thallophytes, bryophytes, pteridophytes and spermatophytes, the first and last groups being represented the most. The thallophyte forms are submersed plants and the spermatophyte mostly land plants. Among bryophytes and pteridophytes are but very few halophytes.

As a contrast to the term *halophyte* the name *glykophyte* (305) has been proposed for plants growing in an environment low in salt content. Species able to grow only in an environment rich in salt are called *obligate-halophytes;* those able to adapt themselves to a glykophytic condition as well are named *facultative-halophytes*.

There are limits to the salt concentration that different species are able to endure, and the result is a zonation among land as well as aquatic species. There are species which have been designated as halophytes because in their natural environments they live in areas rich in NaCl, but experiments (22, 23, 62, 139) have shown that some of them can well be grown in an environment deprived of that salt. Stocker (305) proposes the term *miohalophytes* for them.

Many workers (56, 57, 256, 265, 274, 279, 347, 349) engaged in plant geography, ecology or sociology have divided the halophytes either over the entire world or in their special localities of observation into different formations, associations, *etc.* Sometimes it becomes quite confusing to standardize from among the different authors. Warming (349) distinguishes three types of vegetation or societies (Vereine), namely, aquatic, salt-marsh and halophytic land. The first is divided into a plankton group and a benthos group, each with a number of formations. The former of these he again divides into halo-microplankton, sapro-plankton, and halo-megaplankton. The latter is grouped into haloneides, saprophytes,

higher autophytic algae, and enalids or "sea-grasses." The salt-marsh plants are composed of algae and bacteria, annuals, perennials, half-shrubs, shrubs and trees. The third society has another type of division among its formations: on rocks, on pebbles, on moist sand in the tidal zone, on moist mud soils periodically inundated, and on dry salt-containing soils.

It is of interest to mention also the division of halophytes in Schleswig-Holstein according to Christiansen (57). He divides this flora into a number of orders (Ordnungen) and associations (Verbände). He distinguishes the following orders: Potametalia, Phragmitetalia, Litorelletalia, Junceto botnici-Triglochietalia, and Ammophiletalia.

Those plants that are found in the slits of rocks along the coast are called chasmophytes by Schimper (274). Among such halophytes we sometimes find *Aster Tripolium, Silene maritima, Spergularia marina, Triglochin maritimum, Glyceria maritima* and *G. distans*.

PHYSIOLOGY

Osmotic pressure. Among the early important physiological observations on halophytes (42, 103, 273, 274, 348, 349), one of the first made by Schimper was that increase in salt content in plants increases their osmotic value. Consequently it was thought that the osmotic "pull" of the roots to take up water and its solvents must be considerable. It would make saline soils or waters physiologically dry, notwithstanding the fact that they are physically moist. Therefore, according to Schimper, halophytes are under much the same condition as xerophytes which are inhabitants of physically dry soils, and this similarity was also indicated to him by the fact that some halophytes have fleshy leaves (succulents), others have leathery (sclerophyllous) foliage, and some have reduced leaf surface (aphyllous). Furthermore, he noted water-storing tissues among all groups. The views of Schimper were apparently substantiated, and at first also accepted by men like Warming, but later, other data (68, 142, 143, 155, 264) changed the prevailing views entirely. Chermozon (53) was one of the first who concluded from his anatomical studies that real halophytes could not be considered as xerophytes. Then, due to other work also (79, 80, 214, 215, 305, 309), the views of Schimper as proposed in the first edition of his "Pflan-

zengeographie auf physiologischer Grundlage," could no longer be maintained.

According to Stocker (305), the high osmotic values in the protoplasm of halophytes may be the results of direct action by intracellular salt-ions. According to Kaho (154), cations cause the permeability of protoplasm to be lowered in the following sequence: K $<$ Na $<$ Mg $<$ Ca, while anions cause swelling of the biocolloids and, therefore, increase permeability in the following lyotropous arrangement: $Na_3 > Cl > SO_4$.

Berger-Langenfeldt (28) found that *Honckenya peploides,* a representative of the xero-halic halophyte type, to be described later, has an osmotic value of 5 to 10 atm., whereas *Triglochin maritimum,* a hygro-halic halophyte, has a value of 20 atm. He, as well as others, came to the conclusion that osmotic pressure depends upon the salt concentration in the soil, and he shows in curves that the two types of halophytes follow each other with considerable accuracy. Lundegårdh (195, 196) calculated the pressure in *Suaeda maritima* to be 8.1 atm., in *Crambe maritima* 9 to 13 atm., in *Armeria elongata* 14.3 to 18.9 atm. and in *Atriplex latifolia* 32.1 to 34.6 atm. Uphof (327) studied the osmotic pressure of a number of halophytes along the coral reefs of Cuba, whereas Sen-Gupta (286) did likewise in salt regions of Bengal at the mouth of the Harinbhanga River, and found the following values: in *Aegiceras majus* 38.20, in *Rhizophora mucronata* 32.13, in *Avicennia officinalis* 25.92, in *Ceriops Roxburghiana* 26.65, in *Lumnitzera racemosa* 41.93 and in *Carapa obovata* 39.95, against a water-analysis of 9.845.

According to Lundegårdh (195), halophytes with the highest osmotic pressures grow in the lower littoral zone. *Atriplex latifolia* shows 29.3 to 37 atm., *A. littoralis* over 37 atm., *Aster Tripolium* 26.8 to 32.1 atm., and *Spergularia salina* develops 29.4 atm. In the upper littoral zone where sea water does not cover the ground periodically, the osmotic pressure in cells of *Honckenya peploides* ranges from 12.9 to 18.5 atm., and in *Rumex crispus* and *Solanum dulcamara* it is 18.5. Corresponding differences occur in plants in tropical climates.

Transpiration. Ricôme (263) has shown that transpiration in *Malcolmia maritima* and *Alyssum maritimum* is hardly retarded by NaCl, and that in *Artemisia maritima* it is very strong. Halophytes from the coast of northern Europe show considerable transpiration,

and their structural organization is not of such a nature that it would retard the loss of water, as has been claimed by others (68, 195, 196, 214, 264, 305). Stocker has shown that in relation to the amount of transpiration, halophytes closely approach meso- and even hygrophytes. When the surface transpiration of *Erica tetralix* was placed at 1, of *Calluna vulgaris* at 1.4, of *Cereus* at 0.48 and that of *Sempervivum tectorum* at 0.3, that of the halophytes along the North Sea was between 2.5 and 5.7. Whereas the degree of succulency of cacti is valued between 50 and 300, that of *Salicornia herbacea* is not more than 7.7 and of *Aster Tripolium* 8.6. While water in true desert succulents, as cacti, is typical storage water, the water in halophytes having the appearance of succulents is every day replaced 1 to 2½ times as a result of transpiration. It has been demonstrated that halophytes grown in a substratum rich in NaCl show better regulation in transpiration than when grown in soil poor in salt and supplied with the same amount of water. This is explained as a result of the stomata closing more readily when there is less water taken up by the roots, due to a higher osmotic "pull" in a more concentrated NaCl solution.

Transpiration from mangroves along tropical shores is considerable, sometimes more than that of hygrophytes, but they do not have any xerophytic anatomic construction; their succulency is by no means as pronounced as it was once supposed to be by Schimper. Transpiration in *Sonneratia acida* is 4.2, in *Bruguiera gymnorhiza* 7.4 and in *Rhizophora mucronata* 8 to 8.9. It is worth mentioning that *Statice Gmelini,* an inland halophyte from southern Russia, transpires more water than does *Fagopyrum esculentum* or *Vicia faba.* Keller (162, 164) made similar observations in the semi-arid regions of southern Russia.

There is greater transpiration from halophytes, therefore, than was originally supposed, and this is possible if, as one factor, there is regulation in osmotic pressure. Especially in warm countries there is in plants, such as mangroves, a higher osmotic pressure in the cells at low tide than during high tide, caused by the higher salt content of the soil as a result of strong evaporation. It has been found that the cells of the epidermis and of roots in *Avicennia marina* may have during high tide a pressure of 80 and 50.3 atm., whereas during low tide, *i.e.,* during strong evaporation from the exposed soil, they have 163.2 and 96.6 atm. These differences are less pronounced in cold climates.

Schratz (277) also found that surface transpiration of halophytes is considerable and not infrequently exceeds that of many glykophytes. It is of special interest to note that in halophytic species of the genus *Plantago,* transpiration is higher than that of the glykophytic. According to Holttum (144), *Acrostichum aureum,* a fern from the salt marshes, transpires freely, and the stomata occupy about one-third of the entire lower epidermis. Adriani (2) also studied the difference in transpiration of a number of halophytes in various environments.

Suction pression. Cooper and Pasha (63) pointed out, in their studies in British India on the mangrove vegetation (*Acanthus ilicifolius, Sonneratia apetala* and *Avicennia officinalis*), that suction pressure is greatest in the leaves, medium in the stem, and lowest in the roots. A marked increase could be noticed in each species from August to October. They agree with others (68, 115, 116, 136, 137) that there is in halophytes a more vigorous transpiration than in mesophytes. The former lose more water in the dry season than in the monsoon when the air contains much humidity. There is some possibility that the high suction pressure during the rainless season may be the cause of greater intake of water. Cooper and Pasha conclude that "it is possible that the rise in the suction pressure is brought about by the vigorous transpiration which may bring about greater absorption of salts along with water into the cells."

Salt secretion. Some halophytes are able to secrete salt from the surface of their leaves, and sometimes from their stems through hydathodes. These stomata-like organs are also partly the exit of considerable loss of water. Such secretions prevent too high an osmotic pressure in the cells, and they have been studied by a number of investigators (79, 84, 85, 86, 267, 279). Not only is NaCl secreted but also other salts, as has been demonstrated by Schtscherbak and Ruhland. Not infrequently leaves are covered by a crust of salt as a result. Recently, Mullan (220, 221, 222) studied glandular hairs on both surfaces of leaves in Asiatic halophytes. They are found in pits or are covered by a dense mass of trichomes, and usually each gland is composed of a short stalk-cell and a discoidal or sphaeroidal head with granular contents. They proved to be salt-secreting hydathodes, and salt incrustations were found on the leaves of *Aegiceras majus, Acanthus ilicifolius, Avicennia officinalis* and *A. alba.*

Organs resembling salt glands have also been observed (338) on similar hairs of some psammophilous halophytes, *e.g., Ipomoea pes-caprae, Clerodendron inerme* and *Neuracanthus sphaerostachys.* When grown under mesophytic conditions, these glands develop but little, and their discoidal cells show less granular content. Glands were not observed in *Rhizophora, Ceriops, Bruguiera* and *Sonneratia.*

Amount of salt taken up. Some species of halophytes absorb only as much salt as they are able to withstand at any one time, whereas others take up as much as they are later able to secrete. Keller (160, 163) has demonstrated that in *Salicornia,* belonging to the first group, the amount of NaCl in the plants is considerably less than in the soil. When it is increased 50-fold in the soil there is in the plant an increase of hardly 5 times. On the other hand, when the salt content in the soil is very low, there will be a higher concentration in the cells. Apparently it is taken up until a maximum concentration for the plant is reached. This explains the fact that in some desert regions poor in NaCl certain species are able to gather a considerable amount of NaCl.

Ruhland (267) was able to grow *Statice Gmelini* in solutions containing 10% and 12% NaCl. In the latter instance the supply of water became difficult and there developed danger that the plants would dry out. The pressure in the epidermal cells rose to 165 atm.

Water-storing capacity. Mullan (221) describes the water-storing devices of some halophytes, especially among mangroves and some psammophilous species. The former have a distinct water-retaining tissue, found in the majority of species beneath the upper epidermis, especially in *Rhizophora mucronata, Ceriops Candolleana, Bruguiera caryophylloides, Aegiceras majus, Acanthus ilicifolius, Avicennia officinalis* and *A. alba.* In the psammophilous species a few, *e.g., Ipomoea pes-caprae* and *Scaevola lobelia,* possess a distinct, centrally placed water-storing tissue. It seems that the majority of this group devoid of water-storing tissues, store water in the enlarged cells of the upper epidermis. Schimper considers these water-storing tissues as a guard against the injurious effect of too high salt concentration, whereas Warming considers them as a check against respiration and the penetration of heat rays.

Other important investigations on salt content and osmotic pressure have been made by Keller (165, 166, 167) in semi-deserts of

southern Russia. *Camphorosoma monspeliacum,* which has parts of its roots in the soil layers poor in NaCl, develops others in layers with high salt concentration. The former roots are useful only in spring and autumn when there is an abundance of moisture. During the summer heat and drought, water has to be obtained from lower parts of the soil, rich in NaCl, with the result that the boundary of plasmolysis is between 12% and 14% KNO_3, and during the wet season between 6% and 8% KNO_3.

Germination. Kniep (172) found that in a solution of 0.6 to 0.7% salt no egg cells germinated in *Fucus vesiculosus* and *F. spiralis,* whereas in *F. serratus* they did not develop in a 0.9% solution. The salt content was gradually increased to 3%, and at that concentration *F. serratus* germinated 100%, *F. vesiculosus* 97% and *F. spiralis* 90%. Germination decreased again in *F. serratus* when stronger salt solutions were used; only 3% germinated in a 6% solution and none in 7% solution.

Whereas the influence of sea water on the germination of seeds of halophytes and glykophytes is principally the same, there are differences that occur later between the different groups. In maize, for instance, optimum development is in fresh water, and development is retarded in sea water, whereas with *Salicornia* optimum development takes place in a 1.5% to 3% salt solution, and when the percentage of salt is higher there is diminution of root development until a 6% solution shows the same effect on growth as fresh water. *Aster Tripolium* is intermediate in this respect between the two foregoing plants. Seeds derived from areas of low salt content show a growth optimum of 0.5%, and those from 1.5% salt a 1.5% optimum. The roots from soils weak in salt show an optimum development like those of glykophytes, whereas those from salty places have an optimum of 0.8%. This species may be grown both as a halophyte and as a glykophyte. Important work on the germination of seeds of halophytes has been done by Poma (250) and by Montfort (215).

Other physiological considerations. In a number of glykophytes there is selective absorption of various substances, but this quality has not yet been studied in halophytes. According to Montfort (213, 214, 215) and Brandrup (218), sea water is a balanced solution, and its organisms, as experiments have shown, are not able to live in a pure solution of NaCl or of other salts, any better than do

glykophytes. The same holds for land halophytes, all of which poisoned by pure NaCl solutions when the action of alkali-ions is not removed by additions of Ca-ions.

Halket (116) found that *Salicornia Olivieri* grown without NaCl did not flower, whereas a soil containing 3.4% to 5% salt stimulated the plants to produce flowers. Lesage (188) observed that the glykophilous *Lepidium sativum* became more or less fleshy when the soil was watered with a solution of NaCl. It is also known that *Phragmites communis* and some other species produce not infrequently a low, scanty growth along the shore, and, according to the observations of Keller, *Typha latifolia* does not produce flowers in the Wadi Natrun of Egypt. Carefully conducted investigations are needed to clear up many of these problems.

According to Iljin (146, 147, 149), only those plants should be considered as halophytes whose protoplasm is resistant against a high percentage of salt, especially against Na-ions. Some species are resistant to Na, others to K or Ca, and many that are resistant to Na do not tolerate K or Ca. Cells of glykophytes are easily killed by Na-ions, whereas halophytes not only thrive in a NaCl-solution of considerable concentration but start growing again when put into a NaCl-solution after having been dried out for some time. The ionic action is specific, because these halophytes would die in a weak Ca-salt solution, whereas many a glykophyte withstands a considerable concentration of Ca, but succumbs in a weak concentration of a Na-salt.

Some aquatic halophytes are found in dense formations in relatively high salt concentrations but are absent from adjacent waters that contain slightly less salt. Parts of the sea that do not contain the necessary amount of salt for *Laminaria,* are barren of this genus. Whereas land halophytes may grow at least artificially under glykophytic conditions, typical aquatic halophytes are unable to exist in fresh water. There are, however, some species that form transitions; for example, the *Zostera*-type requires the normal salt content of sea water, the *Ruppia*-type is adapted to fresh water as well as normal sea water, and the *Myriophyllum*-type may grow with a small salt optimum in brackish water.

Stocker (305), too, has shown that some of the views expressed by Schimper were misconceptions. He demonstrated that xerophytic properties among halophytes were much overrated by Schimper

who presumed that in the mangroves which he studied in Java, xeromorphy serves to prevent accumulation of NaCl in the leaves, and that otherwise there would be, as a result of high temperature combined with great absorption of water, so much salt in the organs that it would become harmful to the plant. Experiments of Stahl (301) have proven that this hypothesis can not be maintained.

MORPHOLOGY

Morphological studies (53, 128, 196) have shown that plants from an environment rich in salt show a tendency toward succulency by having thicker leaves and stems, and that there are a more pronounced palisade parenchyma, smaller intercellular air spaces, and often diminution in the number of chloroplasts. These characteristics are not general, however. Among 85 species Lesage found 54 in which succulency of the leaves developed under halophilous conditions as compared with individuals grown under glykophilous conditions; 27 species, among which were typical halophytes including *Glyceria maritima* and *Suaeda maritima,* remained unaffected; and 4 showed less succulency. Lesage (188) experimented with *Pisum sativum, Linum grandiflorum* and *Lepidium sativum,* all glykophytes, and observed that NaCl reduced their length, caused thicker leaves, strengthened the palisade parenchyma, and reduced the intercellular air spaces and the chlorophyll content. Keller (160, 163) found that in carefully selected salt solutions *Salicornia herbacea* succulency increased and the surface became reduced. The causes of these differences are still not known, but, according to Batalin (22), who experimented with *Salicornia,* they appear to be responses to the salt solution.

GENETICS

There is a paucity of investigations based on modern genetical research which might clarify some of the problems concerning the physiological peculiarities and morphological characteristics of halophytes. A few data, however, may be considered under this topic.

Christiansen (56, 57) sets forth the possibility that some species have given rise to several halophilous races (Salzrassen), and it can easily be observed that many species growing near the shore have particular characteristics entirely different from those of closely related species growing in a purely glykophilous environment. We

distinguish along the shores *Armeria vulgaris* var. *maritima, Anthyllis vulneraria* var. *maritima, Atriplex hastatum* var. *salinum, Bromus hordeaceus* var. *Thominii, Euphrasia rubra* subsp. *litoralis, Viola tricolor* var. *maritima, Taraxacum officinale* subsp. *palustre* var. *balticum, Weingaertneria canescens* f. *maritima, Matricaria inodora* f. *maritima, Polygonum aviculare* var. *litorale* and *Jasione montana* f. *litoralis* besides a number of other varieties whose morphological and physiological variations might have arisen under the influence of an halophilous environment, or that might have originated among a number of random mutations able to survive only in their new environment. There is, furthermore, no geographical connection between a number of varieties and subspecies along the North Sea and others along the Baltic. It is likely that they belong to different races, each with its own origin, North Sea races from western Europe, and Baltic Sea races from eastern Europe and Siberia. For example, along the North Sea *Artemisia maritima* subsp. *maritima* is prevalent, whereas *A. maritima* subsp. *salina* often occurs abundantly along the Baltic. Furthermore, *Cakile maritima* var. *normalis* is a North Sea type, whereas along the Baltic coast there is *C. maritima* var. *amblycarpa.*

CYTOLOGY

Wulff (360) made a series of cytological studies on the number of chromosomes of 30 species of halophytes in Schleswig-Holstein. Following the suggestion of Hagerup that among angiosperms growing in an unfavorable climate there is a high percentage of polyploid species, he found that in *Obione portulacoides* $2\ n = 36$; in *Suaeda maritima,* 36; in *Salsola kali,* 36; in *Salicornia herbacea,* 38; in *Althaea officinalis,* 40 to 44; in *Glaux maritima,* 30; in *Erythraea litoralis,* 32; in *Statice Limonium,* 36; in *S. sinuata,* 18; in *Scirpus rufus,* 40; in *S. americanus,* 80; in *Juncus Gerardi,* 80; and that *J. ranarius,* which is considered by some as a halophilous variety of *J. bufonius,* has $2n = 108$ and 120, whereas *J. bufonius* has at least 20. There are, however, a number of other halophytes that suggest a diploid number of chromosomes; among these are *Artemisia maritima* with 18, *Atriplex litorale* with 18, *Plantago maritima* with 12, *Najas maritima* with 12, and several others with diploidy. Among the 60 halophytes and semi-halophytes were 29 with diploid number and 31 that were polyploid. Both occur in practically the same

ratio, and there is no indication, therefore, that unfavorable conditions result in polyploidy.

SALT CONTENT IN SOILS AND WATERS

Halic moist land areas. The concentration of NaCl and other salts in the soil is difficult to measure, and significant averages, as those obtained from the sea or salt lakes, can not be given. It may be readily presumed that soil water near the coast is usually influenced by the salt of the sea, but that when further removed, rain water, fresh water from streams, and various subterranean currents, no matter how small, must have considerable influence upon the concentration of salts in it. Important variations, therefore, must occur in the smallest areas and in the smallest amount of time, which can not be measured. According to Stocker (309), a certain part of the sandy shore covered with *Triticum junceum* and *Honckenya peploides* contained 0.2 to 0.3% salt, whereas the nearby water of the North Sea (near Cuxhaven) contains 3%.

Clay soils can hold more salt than can sand, and it is not so easily removed by streaming fresh water. This results in a zonation of species. A zone of *Salicornia* can be distinguished which is frequently moistened by the sea water, a *Glyceria maritima*-zone where high tide exerts its influence but rarely and then usually for a short time, and a *Festuca rubra*-zone which seldom comes in touch with the sea water. Salt concentration becomes considerably increased during high transpiration, especially in summer, and particularly in tropical countries. Stocker found that the soil of a *Salicornia*-zone may contain as much as 6% salt, and a mangrove zone, according to von Faber, may contain to 12%. Also the distribution of salt particles by wind must be considered, a theme which was studied by Häyren (132) and Ginzberger (104).

Arnold (13) investigated various soil conditions, especially of the East Frisian Islands, and found that the salt content of the sandy beach is never higher than that of the adjacent sea water. The salt is gradually leached out vertically by the rain and is then removed horizontally with fresh water coming from the interior.

Halic dry-land areas. There are land areas containing salt in several countries and at different elevations. The concentration varies up to the saturated condition and to crystallization of various kinds of salts. High concentration is usually caused by high evap-

oration together with inadequate drainage, and the salt content remains, therefore, more or less the same. Halic dry-land areas are found mainly in desert regions, where the upper soil often contains a considerable amount of salt, whereas at a depth of two meters or more there may be considerably less. In such regions many plants have been found which penetrate to that depth, thus being less halophytic than plants of the upper soil. *Alhagi camalorum*, for example, growing in salty deserts of Turkestan, has such deeply penetrating roots that they extend below the level of concentrated NaCl, and this species, therefore, hardly deserves the name of halophyte. On the other hand, *Salicornia* and *Salsola*, growing in the same region, have root systems that remain near the upper layers of the soil in a true halic environment.

Salt content in salt desert regions is not uniformly distributed, horizontally or vertically. In some localities, as the south Russian semi-deserts near Krasno-Armeisk, the salt content is higher in the lower strata of the soil. In some of the Egyptian rocky deserts the reverse is the case. During the rainy season when the salt concentration becomes less, the conditions for germination are generally more favorable. In most salt deserts the salt crystallizes on the surface during the dry season, and many such regions become swamps (in some localities called takyrs) during the rainy season.

Seas and oceans. NaCl is the most abundant salt in sea water. Generally speaking there are about 27.213 mg. of NaCl dissolved in 1000 grams of sea water, followed by 3.807 mg. $MgCl_2$, 1.658 mg. $MgSO_4$, 1.260 mg. $CaSO_4$, .863 mg. K_2SO_4, .123 mg. CaCO and .76 mg. $MgBr_2$. There is considerable difference, however, in the salt content of different parts of the ocean. The water of the Baltic Sea contains 1 to 3%, the Arctic Ocean 3.4 to 3.5%, and the Atlantic Ocean between 3.5 and 3.6%. The highest amount known, from the Persian Gulf, is 4%. There is also a difference in the salt content of the different layers of the sea. Near Romereimsfjord Nordgaard (232) found 1.06% near the surface, 2.39% at a depth of one meter, 2.98% at three meters, 3.06% at 5 meters, and 3.42% at a depth of 20 meters. In spring there is a 10-meter-thick upper layer of water of less salt content moving from the Baltic Sea through the Skagerrak, while there is an undercurrent of water from the ocean moving in the opposite direction with a much higher content. This has considerable influence upon some Phaeophyceae, as has been demon-

strated by Oltmanns (236, 240) and Montfort (215). When the salt content diminishes, *Fucus serratus, F. vesiculosus* and finally *F. Sherardi, Ascophyllum nodosum* and *A. ceranoides* gradually disappear.

Inland seas, lakes and marshes. Very high concentrations are found in the waters of some inland seas and lakes. The Wadi Natrun in Egypt may contain, according to Stocker (308), up to 37.8% of salt. This high concentration is reached in spring and summer when the supply of fresh underground water, at other times flowing into the salt swamps, diminishes.

Some one-celled algae of *Chlamydomonas* are able to thrive in a concentration of 2 to 25% NaCl. As a rule, development of organisms is impossible when the concentration reaches about 20%, and this can be noticed in parts of the Dead Sea. On the other hand, some organisms are able to live where the concentration is 10 to 15%. Woronich (359) found that in Tambukan Lake in northern Caucasia different species of algae had accumulated in water containing 11½% to such an extent that they made travelling by boat difficult.

The water of the North Sea which influences surrounding salt marshes contains near Heligoland 3½% NaCl whereas fresh water on the mainland nearby contains but .02% in an area where only glykophytic associations are maintained. Arnold (13) found that the ground water of the island of Wangeroog may contain as much at 9%. Soil analyses have demonstrated that in a small space there may be considerable fluctuations in the concentration of salt. In some regions, like that around the Dead Sea in Palestine, the salt content is so highly concentrated that the soil can support but very little vegetation. Adriani (1, 2) studied the changes in salinity during summer months in a number of communities in a salt desert of the French Mediterranean. During the summer the salt concentrates to such an extent that it finally crystallizes. The same may be found in other localities. That some of these areas support considerable vegetation proves the wide range of salinity certain halophytes are able to endure. Flowers (87) studied the behaviour of ions in the Great Salt Lake, Utah, especially in relation to sodium and calcium. He found that calcium carbonate is deposited on sand grains, forming oolite, or upon groups of algae, especially *Aphanothece* and *Microcystis,* giving rise to tufa-like deposits. The algae

accumulate in the shallow water and become cemented down to the increasing bed of tufaceous deposit which is especially visible along the shores of some islands. The main body of water of the above mentioned lake varies from 15.71 to 21.70% salt. The pH value ranges from 8.2 to 8.6. The salt content at some bays, swamps and inlets of this lake amounts to 1.03, 5.86, 10.60 and 11.68%.

DISTRIBUTION OF HALOPHYTES IN THE PLANT KINGDOM

All four major divisions of plants have members that are adapted to halophilous habitats. Thallophytes have the largest number. The Rhodophyceae and Phaeophyceae, with very few exceptions, are entirely adapted to a halophilous life. On the other hand, the percentage contributed by the Chlorophyceae is considerably less.

Lichens

There are a few species of lichen that are adapted to life near the sea. It is of interest to note that other species considered as related to many of those near the sea are found inland far removed from the coast. The occurrence of some along the coasts of Scandinavia and Finland has been studied (94, 95, 132, 288, 289), and Tobler (320) gives a general review of the problem. There is among these species growing on rocks, a specific and often high degree of adaptation toward the salt water and sea winds. A broad field of study still lies open in these symbionts, as well as in their individual components. Close to the sea, in the supralittoral region, the rocks are covered by *Verrucaria maura;* groups of this lichen are especially developed toward the north side of the rocks. The plants are continuously moistened by the waves, and that part of the rocks which is continually wetted by fine spray (Spritzgürtel of Häyren) is divided into two zones, the lower on which *Caloplaca murorum* is dominant, and the upper which is taken by *Rhizocarpon geminatum.* The boundary zone (Grenzgürtel), less sprayed by the mist of sea water, is covered by *Parmelia prolixa.* The supra-marine zone, above the spray zone but subject to sea winds and fogs, is covered by *Ramalina scopulorum, R. sulfarinacea* and *R. cuspidata.* The supra-marine inland zone above the spray zone is often gray from a covering of *Parmelia saxatilis.*

Mycorrhiza

Mason (205) and Klecka and Vukolov (171) found that meadow halophytes possess mycorrhiza, especially *Samolus Valerandi, Su-*

aeda maritima, Salicornia herbacea, Plantago maritima, Carex secalina, Triglochin maritimum, Aster Tripolium and *Juncus Gerardi.* The fungi involved are identical with the endotropic mycorrhiza of woody plants, penetrating the intercellular spaces of the cortex and rolling up there into balls and breaking into irregular pieces surrounded by cell protoplasm. This is considered as a digesting process, and all the kinds involved seem to share the quality of enduring the high osmotic pressure of the root cells.

Bryophytes

The number of halophilous bryophytes are also few. Herzog considers as typical halophytes the following species: *Pottia Heimii, P. crinita, P. salina, P. commutata, P. propagulifera, Schistidium maritimum, Bryum salinum* and many other species of this genus, *Tortella flavovirens, T. viridiflava,* and *Petalophyllum Ralfsii.* According to Gams (97), certain leptobryeta and funarieta pass to typical halophytes with *Pottia Heimii, P. crinita, P. salina, P. propagulifera, Bryum salinum, B. calophyllum, B. Friderici-Mülleri* and others associated with halophilous phanerogams, such as *Atropis.* Some of these halophytic bryophytes are also found in saline places far inland, for examples, *Pottia Heimii* and *P. crinita,* whereas *Ulota phyllantha, Grimmia maritima* and a number of *Bryum* species do not leave the coast, according to Monkmeyer (212). *Pottia Heimii* is distributed on saline soils from the temperate zone to the arctic. *P. crinita* is found along the coast of northern France and England as well as inland. *Grimmia maritima* grows on siliceous rocks along the coast of the North and Baltic Seas and as far as the Arctic. *Swartzia inclinata* and *Bryum nitidulum* have been found near Spitzbergen associated with *Carex subspathacea* on the edges of mud flats, but it does not survive below the limit of normal high tide.

Pteridophytes

There are few pteriodophytes which grow on saline soils. None of them is an obligate halophyte. The most conspicuous is *Acrostichum aureum,* widely distributed in the tropics and some parts of the subtropics. This species starts its distribution, so far as the New World is concerned, in the southern half of Florida. It is frequently found in salt marshes forming, according to Uphof (332), dense

clumps where other species are not able to penetrate. It is of frequent occurrence in many mangrove formations of both hemispheres. This characteristic pan-tropic fern is found also in the drier parts of the mangrove formation, where it is often subjected to high tides, as well as in brackish and fresh-water swamps and along rivers, though usually not far from the coast. The writer found it in similar localities of Cuba, Honduras, Guatemala and El Salvador whence it is distributed along the tropical coast of South America. *A. danaefolium* occurs in brackish swamps though it is found mostly in a glykophilous environment. Also *Humata parvula* is known in a similar environment.

Marine Spermatophytes

Spermatophytes able to adapt themselves to marine life belong to either the Hydrocharitaceae or the Potamogetonaceae. Among the former are *Halophila, Enalus* and *Thalasia,* and among the latter *Zostera, Cymodocea, Diplanthera, Phyllospadix* and *Posidonia.* All comprise about 40 species. The majority, except the littoral *Phyllospadix,* live in shallow waters, hardly over 10 fathoms in depth, and form the upper sublittoral belt. They are seldom uncovered by ordinary low tides. Many investigations of these plants have been conducted (75, 197, 209, 244, 246, 290). As is true of other submersed plants, they live in a greater uniformity of temperature than do land plants. The majority produce long prostrate rhizomes buried in sand and mud. *Posidonia oceanica* and species of *Phyllospadix,* on the other hand, are found in rocky or stony environments, and form relatively short, condensed, often somewhat erect rhizomes. Most species, being strictly stenothermal, are, according to Setchell, "confined strictly to one temperature zone of 5° C. amplitude of the monthly mean maximum of the surface waters." Some species extend over two zones of temperature, one of which may be considered as normal for them and the other as one to which they have become adapted. *Zostera capricorni* and *Cymodocea antarctica* may be given as examples of the latter group. The first of these is found from Cape York in tropical eastern Australia to Port Jackson in subtropical eastern Australia; the latter species occurs from the western and southern coast of Australia to the northern shores of Tasmania. *Halophila ovalis* and *Zostera marina* are found in three or more zones. The first of these grows in the Red Sea, the Indian Ocean

and tropical parts of the western Pacific, around Australia and as far as Tasmania. The second species is confined to the northern hemisphere, extending from the arctic to the tropics along the coasts of the Atlantic and Pacific; it is, however, rare in both the frigid and tropical zones.

SALTBUSHES

The name "saltbush" applies to a number of species having a salty taste, many among them being used as food by cattle, usually under adverse conditions where they are valuable as drought-resistant fodder plants. Many belong to the Chenopodiaceae and are, therefore, related to each other. Some are shrubs, others herbaceous plants. The majority are halophytes and have been mentioned elsewhere in this contribution. Several species are native to the semi-arid regions of the Southwest; others are found in parts of Australia, South America and elsewhere. Some of the "saltbushes" as described by Bidwell (34) are: *Allenrolfea occidentalis, Atriplex acanthicarpa, A. argentea, A. canescens, A. confertifolia, A. leptocarpa, A. Nuttallii, A. semibaccata, Chenopodium arizonicum, C. cycloides, C. salinum, Dondia suffrutescens,* and species of *Endolepis, Eurotia, Grayia, Kochia, Sarcobatus, Amaranthus, Gomphrena, Eriogonum* and *Rumex*. Together, they all constitute an artificial rather than a natural group.

DOMINANCE IN OPEN SEAS

Halophytic flora is sometimes quite dominant in the open sea, but very little work has been done on the nature of the dominance. During laboratory experiments *Skeletonema costatum* in water from the open sea always displayed extreme dominance, for when it died others increased. *Nitzschia seriata* has been distinctly dominant during some summers at certain points near Santa Barbara Island, California, and its dominance is supposed to be due to the influence of some animal. *Chaetoceras curvisetum* shows dominance in April in the San Diego region, whereas *C. criophilum* in a typical oceanic environment is dominant in June and July. Allen (3), who observed that swarms of diatoms and dinoflagellates almost exclusively occupied whole areas of a few square yards to several hundred square miles in extent, suggests that this dominance may be due partly to an ability "to develop some offensive secretion or deterrent activity which enables it to drive out and

displace other plants." Some dominant species may be entirely carried away by a wide sweeping storm current or they may be destroyed by ocean water of another temperature from an upwelling current, a change in pH, or predatory animals.

DISSEMINATION OF FRUITS AND SEEDS

Fruits and seeds of most coastal halophytes are exceedingly well adapted for dissemination by the sea, and the world-wide distribution of certain species is attributable to this condition. There is no doubt that ocean currents, the Gulf Stream, for example, aid considerably in such distribution. Warburg (345) and Schimper (273) have studied this matter and have shown that most species so distributed contain large intercellular air-spaces in different organs which enable them to float for considerable periods of time. *Gyrocarpus Jacquini* has large lacunae within the seeds where the cotyledons lie with only one side directly against the seed-coat. *Entada scandens* shows lacunae between the cotyledons. *Canavalia obtusifolia* has sponge-like large intercellular spaces in the cotyledons. *Suriana maritima* has lacunae in the seeds. *Thespesia populnea* shows hollow parts between the fruit and the seeds. *Scaevola Lobelia* has aerenchymatous tissue in the endocarp. *Ipomoea pes-caprae* and *I. carnosa* show small hollow spaces in the seeds. *Cocos nucifera* has an extensive floating tissue in the mesocarp. In *Batis maritima* the fruits become dry after the seeds have ripened, and therefore very light and suitable for distribution by the sea.

It was found that seeds of *Hibiscus tiliaceus* were still able to float after 120 days in a $3\frac{1}{2}\%$ NaCl solution, and *Suriana maritima* after 143 days. *Euphorbia Atoto* still floated after 4 to 5 days and the seeds of *Canavalia* after 70 days.

GEOGRAPHICAL DISTRIBUTION

Arctic. According to Tolmatchew (321) all beach halophytes gradually diminish in size within the arctic, and the number of species dwindles. Apparently, *Halianthus peploides* is the most widely distributed, for it is found on Iceland, Jan Mayen and Spitzbergen, as well as along the coast of northern Scandinavia and that of the White Sea and similar areas. He concludes that *Mertensia maritima* has a similar distribution. Within the arctic there are

also *Plantago maritima, Lathyrus maritimus, Chrysanthemum arcticum, Carex subspathacea* and *Puccinellia phrygamnodes.*

The vegetation of the coast line of Iceland is composed of a few species. This coast, according to Thoroddsen (319), consists of fine and coarse sand, pebbles and boulders; at some places there are rocks of basalt and of tuff. Upon the low rocks there is a belt of *Verrucosa maura* which is followed by a number of other species of lichen. Above this stratum are mainly *Cochlearia officinalis, Plantago maritima, Glyceria distans* and *Armeria maritima.* On steep coast cliffs there is an abundance of *Cochlearia officinalis, Rhtodiola rosea, Silene maritima, Armeria maritima* and *Cerastium alpinum.* The sandy beaches, especially along the south of Iceland, support *Halianthus peploides, Atriplex patula, Cakile maritima, Stenhammeria maritima, Potentilla anserina* and a few others; *Cakile maritima* grows closest to the sea, whereas *Potentilla anserina* is found at the highest elevations. Most characteristic of Iceland's salt marshes, among others, are *Glyceria maritima, Plantago maritima, Stellaria crassifolia, Triglochin maritimum, Juncus bufonius* and *Heleocharis uniglumis.*

Walton (343) and Dobbs (72) were able to study the halophytic conditions of Spitzbergen. Near Cape Napier the vegetation on mud flats subject to tidal inundation is very limited. There are a few species of Cyanophyceae. *Puccinellia* and *Swartzia* are the most frequent colonizers on the bare mud. The blue-green algae form a thin layer which becomes wrinkled when dry and readily invaded by *Puccinellia. Carex subspathacea* occurs in almost pure masses on silty places above normal high tide. *Puccinellia phrygamnodes* is able to withstand frequent tidal inundations. *Swartzia inclinata* forms a distinct zone outside that of *Carex subspathacea,* playing a secondary role as a colonizer with the bryophytes *Swartzia* and *Bryum nitidulum;* it is unable to survive below the limits of normal high tide. As a semihalophyte *Dupontia Fisheri* may be mentioned, spreading along the mud and shingles within the limits of tidal waves. This member of the Graminaceae is not found far from the salt water and follows *Carex subspathacea* in tolerance of salinity.

Northeastern American continent. The algal flora along the northeastern coast of North America, according to Harshberger (122–129), is composed of different strata or shelves. Among

these is the *Ulva*-shelf; *U. latissima* at some places is very abundant, especially where water is left by the tide. The *Fucus*-shelf is formed by *Ascophyllum nodosum,* and in pools of its depth occur *Chondrus crispus, Chordia flagelliformis, Cystoclonium purpurescens, Hypnea musciformis* and others. The *Laminaria-Chondrus*-shelf is composed of *Laminaria digitata, L. fusca, L. phyllitis, L. saccharina, L. dermatodea, Agarum Turneri* and *Alaria esculenta,* whereas *Chondrus crispus* is found at a greater depth. These are succeeded by the deep sea-shelf of *Laminaria longicruris, Euthora cristata, Delesseria sinuosa, D. alata, D. denticulata, Ptilota serrata, P. elegans, Polysiphonia fibrillosa* and other algae.

The most typical association on areas composed of newly formed salt marshes from the lowest high-tide marks upward is the *Salicornia-Suaeda*-association where *Salicornia herbacea* and *Suaeda linearis* are the principal members. Where it is somewhat drier, as in some parts of the New Brunswick area, and where the land is slightly higher, one finds *Spergularia borealis* and *Atriplex hastata.* High salt marshes only occasionally under high tides are often composed of a *Statice-Spartina juncea*-association. In this association there are besides *Statice caroliniana* and *Spartina juncea, Plantago maritima, Puccinellia maritima, Festuca ovina, Juncus Gerardi, Triglochin maritimum, Hordeum jubatum* and *Glaux maritima.*

One of the first pioneers in salt marshes of the lower littoral of temperate eastern North America is *Spartina glabra* which is usually found along flat banks (227, 302, 329). The next stage is formed by salt meadows composed mainly of *Spartina patens* var. *juncea* and *Distichlis spicata* with *Juncus Gerardi* along the margin. Here and there among these dominant species are *Salicornia herbacea, S. mucronata, Statice caroliniana, Atriplex hastata, Triglochin maritimum, Plantago decipiens, Aster subulatus, Gerardia maritima* and, toward the margin of the marshes, *Aster tenuifolius* and *Solidago sempervirens.* More inland the *Juncus*-zone is often succeeded by *Iva frutescens* and finally by *Panicum virgatum.* Where water becomes brackish the marshes support a number of meso-halophytes, *e.g., Agrostis alba* var. *maritima, Spergularia marina, Potentilla anserina* var. *pauciflora, Gerardia maritima, Pluchea camphorata, Iris prismatica, Hibiscus moscheutos* and species which succeed from the fresh-water swamps into brackish

areas, such as *Typha angustifolia, Scirpus americana* and *Phragmites communis.*

Steiner (302) studied the osmotic value of the soil surface and pH values along the boundaries of these different associations of salt marshes. He came to the conclusion that the hygro-halophytic associations are distinguished from the hygro-glykophytic by higher osmotic values; meso-halophytes and plants of the sand dunes show values related more to glykophytes than to euhalophytes.

Brooks-Knight gives us an insight into the theoretical constructional history of a typical New England salt marsh. In his salt-marsh studies on Long Island Sound at Killan's Point, Conn., he considers an area that has preserved the thus far unrecorded early stages of salt-marsh formation in New England which substantiates Shaler's theory. Killan's Point seems to offer definite field evidence of early stages of marsh formation, coupled with evidence of the effects of a steady rise of sea level. According to Brooks-Knight, the Shaler and Mudge-Davis theories are valid, though each for a different stage of development of the same salt marsh. He considers the New England salt marshes through-and-through Mudge-Davis marshes. Apparently, most began as Shaler marshes. There are along the New England coast numerous marginal marshes protected from wave action by beaches of sand. shingle or cobbles. In this way salt-marsh grasses grow under considerable protection from the waves. *Spartina stricta* is found at lower levels than *S. patens* and, according to Brooks-Knight, it will grow first where the bottom has arisen to the requisite height above tide level, that is, close to the shores of the lagune; the marsh mass will commence its growth in these portions and advance out over the mud flats as they are raised to the requisite level, with *S. stricta* below and *S. patens*-association above, which is the essence of the Shaler theory.

More southward along the Atlantic Coast there is a series of other ecological and sociological compositions among the halophytes. We will consider first the southern limit of Florida. The algal flora there, according to Taylor (317) who studied the Dry Tortugas, indicates typical tropical floral elements. Less than 10% of the species are found near Cape Cod. The salinity of the water varies from 3.1% to 4.1%. Generally, the sandy beach shows but little vegetation, except those algae that have been

washed ashore. Of more importance is the vegetation among the coral reefs. Loggerhead Key may serve as an example. The number of species of Chlorophyceae, Phaeophyceae and especially Rhodophyceae is considerable. Below the low-tide mark is an important development of *Laurencia obtusa, Dilophus alternans, Halimeda scabra* and especially of *Padina Sanctae-Crucis.* Above low-tide mark are species of *Jania, Centroceras, Coelothrix, Padina, Gelidium, Dictyota* and *Sphacelaria.* At the upper limit of plant growth the rocks are almost bare; there are some patches of *Dichothrix olivacea* and a species of *Enteromorpha. Wrangelia Argus* and *Laurencia intricata* are on some rocks, and at a depth of 4½ to 7½ meters are *Amphiroa Tribulus* and *Cladophora fuliginosa,* and species of *Sargassum,* Dictyotae and Galaxaurae. Some species clearly form zones and belts. Among the marine spermatophytes is *Thalassia testudinum* at Dry Tortugas, the most common species, forming extensive beds from near low-tide level to a depth of 11 meters. *Holodule Wrightii* does not go deeper than 11 meters and is luxuriant in some pools. Equally as abundant is *Cymodocea manatorum. Halophila Baillonis* was found to a depth of 29 meters, and *H. Engelmanni,* according to Taylor, at a depth of 4.6 to 73.2 meters.

As far as land halophytes are concerned, zone formation is at most places very distinct. Salt marshes subject to low tide are usually densely covered by *Salicornia Bigelowi* and *S. ambigua,* common on mud flats and sandy places. Usually a little higher there is *Batis maritima,* a species typical of subtropical and tropical shores of the New World and of some of the Pacific Islands (328, 330, 332). Its geographical distribution starts in North Carolina, and it becomes more abundant in warmer regions. *Juncus Roemerianus* is especially plentiful in muddy places where it covers large surfaces and is an important plant in holding the first accumulations of mud. *Distichlis spicata* is the most common salt-meadow grass, whereas on slightly less haline areas are extensive groups of *Acrostichum aureum.* In the neighborhood are often found *Iva frutescens, Borrichia frutescens, Baccharis halimifolia* and *Lycium carolinianum.* Mangrove formations are very numerous and are described elsewhere.

West Indies. Special formations of flowering plants are to be observed along the coral reefs of the West Indies. There is not

only the sodium chloride factor but also the presence of calcium carbonate of which the corals are mainly composed. Uphof (327) has studied these plant formations along the northern coast of Cuba. Many characteristic species of algae grow between the tide marks. Among the pioneer spermatophytes on the coral shores are *Salicornia herbacea* and *S. perennis.* Somewhat higher in the small saline marshes between the coral rocks where some mud is able to accumulate are patches of *Distichlis spicata,* frequently subject to high tide. At a distance of from ½ to 6 meters from the shore are *Sesuvium microphyllum* and *Philoxerus vermicularis.* Where the reefs become a little higher there are *Conocarpus erecta* and *Borrichia arborescens,* and where the salt is able to reach the vegetation only moderately there are small bushes, *e.g., Tournefortia gnaphaloides, Suriana maritima* and *Coccolobus uvifera,* succeeded by shrubs and herbs of glykophilous character.

Atlantic Coast of South America. Lindeman (189) mentions *Salicornia Guadichaudiana,* some species of *Atriplex,* and *Sesuvium portulacastrum* along the coast of the Rio Grande do Sul. There are also associations of *Spartina glabra. Cotula coronopifolia, Althernanthera achyrantha* and *Heliotropium curassavicum* occur on drier localities. Von Wettstein (353), who studied the sandy shore of Brazil, states that there is much similarity between the vegetation of that shore and that of tropical shores of other parts of the world. There are, for instance, the *Ipomoea pes-caprae*-formation, as well as *Canavalia obtusifolia, Stenotaphrum americanum, Remirea maritima, Acicarpha spathulata, Iresine portulcoides* and *Althernanthera maritima.*

Islands of the Pacific. The Galapagos Islands, which have been of great interest to biologists, support an extensive mangrove vegetation. Stewart (304) made an extensive study of the entire vegetation. He observed a number of halophytic and semi-halophytic species along some sandy beaches, *e.g., Cryptocarpus pyriformis, Heliotropium curassavicum, Ipomoea pes-caprae* and *Scaevola Plumieri.* On Barrington Island some bushes of *Cryptocarpus pyriformis* and mat-like growths of *Sesuvium Edmonstonei* are to be seen, though they are not exclusively halophytic. Along some sandy beaches there are *Batis maritima* and *Sesuvium portulacastrum,* whereas elsewhere are *Cacabus Miersii, Coldenia fuscus, C. Darwinii, Discaria pauciflora* and *Vallesia glabra.* On some places the shores are deep and rocky and do not support any halophytes.

The halophytic flora of the Hawaiian archipelago was studied by MacCaughey (198). Being the most northern group of the Polynesian islands they are of importance, though remarkably similar from island to island and from atoll to atoll. Of the endemic littorals, MacCaughey mentions *Lycium sandivicense, Wikstroemia Uva-Ursi, Scaevola coriacea, Noto trichum humile, Phyllostegia variabilis* and *Santalum Freycinetianum* var. *littorale* among the woody plants, and *Achyranthes splendens, Campylotheca molokaiensis, Lipochaeta connata* var. *litoralis, L. succulenta, Schiedea globosa, Lepidium owaihiense* and others among the herbaceous. There are several also found elsewhere among which may be mentioned *Batis maritima, Ipomoea pes-caprae, I. glaberrima, Scaevola Lobelia, Lepturus repens, Boerhaavia diffusa, Heliotropium curassavicum, Herpestis monniera* and *Sesuvium portulacastrum.* I was able to observe a number of these plants along the Pacific Coast of Guatemala and El Salvador in Central America.

Pacific Coast of North America. Along the coast of California and northward some rocks support a considerable amount of *Fucus fastigiata, F. serrata, Plumaria elegans, Corallina officinalis, Cladophora rupestris* and other algae. That part of the beach which lies above flood tide is characterized by a number of herbaceous plants and a few that are woody and of frequent occurrence. One is able to distinguish *Franseria bipinnatifida, Abronia umbellata* and *A. maritima.* There are a number of *Salicornia*-species, *e.g.*, *S. ambigua, S. subterminalis, S. pacifica, S. Bigelowii* and *S. depressa.* They are common in salt marshes and are at several places the first colonizers. In other parts of these marshes is *Suaeda californica. Jaumea carnosa* occurs also on mud flats from Coronado Beach to British Columbia. *Juncus acutus* and *Monanthochloë litoralis* occur from Santa Barbara southward to tropical America. *Scirpus riparius* grows in marshy places from San Diego to British Columbia. *Spartina leiantha* is distributed in salt marshes along the coast to San Francisco. *Statice mexicanum* is found from San Diego to Lower California. *Lasthenia glabrata* forms saline meadows and marshes. *Chenopodium hastata* grows on moist saline places from southern California to British Columbia, whereas *Batis maritima* is to be noticed along the seashore from about Los Angeles southward to South America and the islands of the Pacific. Along the coast the low-growing *Astragalus pycnostachyus* is of

common occurrence. *Sesuvium verrucosum* grows often on saline flats.

Pacific Coast of South America. The long coast along Chile has been well described by Reiche. The regions about Coquimbo and La Serena are covered by large areas of *Salicornia peruviana* forming salt marshes and belonging to the first colonizers. On more established situations grow *Cotula coronopifolia, Selliera radicans* and *Statice chilensis,* whereas *Juncus acutus* forms large patches and is often accompanied by *Baccharis pingraea.* Along the drier regions less subject to the tides are *Frankenia chilensis, Tetragonia ovata, Cristaria urmenata, Mesembryanthemum chilense* and others, and *Distichlis thalassica* may cover large areas. Along the brackish waters of the Rio Loa are dense bushes composed of *Baccharis petiolata,* and directly along the waterfront are *Baccharis juncea, Atriplex atacamense* and *Tessaria absinthoides* with, here and there, *Scirpus glaucus* and meadows covered by *Distichlis.*

Antarctic and Subantarctic—Falkland Islands and Patagonia. Skottsberg (294–297) mentions that in the Falkland Islands the stones and rocks along the coast near the high-water zone are covered with lichens. On the salt meadows there is abundant development of *Deschampia antarctica, Aira praecox, Juncus scheuchzeroides* and *Scirpus cernuus* var. *brevis* with, here and there, some individuals of *Galium antarcticum* and a few of *Atropis Preslii, Marsippospermum grandiflorum, Sagina procumbens, Plantago barbata, Crantzia lineata* and a few other species. In South Georgia, according to the same author, there is along the flat coast and especially along places covered by sand and pebbles, one of the most remarkable plants of the sub-antarctic: *Poa caespitosa.* Along the beach this plant is often surrounded by *Aceana adscendens. Colobanthus subulatus* thrives in the narrowest clefts. *Amphiloma dimorphum* covers rocks as far as the high-tide mark. There is a typical zonation of *Macrocystis pyriforme* in the water along the coast, filling shallow bays with a dense mass between which a few other algae grow. *Ulva latissima* and *Enteromorpha Novae Hollandiae* grow abundantly on the rocks.

North Sea and Baltic Sea coastal areas. Submersed meadows of *Zostera marina,* covering several thousand hectares, are characteristic of several regions of the North Sea. They have been studied extensively by van Goor (111, 112), and occur on places where the

water level is lowest. They are least developed in deeper parts of the sea because of streaming of the water and low intensity of light. According to van Goor (111), *Zostera*-associations are found along certain parts of the coast of the Netherlands where the water contains 3.3% to 2% of NaCl. In these meadows a certain number of Rhodophyceae have been found, among which are *Ceramium rubrum, C. strictum, C. Areschougii, Polysiphonia violacea, P. nigrescens* and *Melobesia Lejolisii,* and in the deeper strata *Corallina rubens* and *C. officinalis.* Species of Phycophyceae occur, according to van Goor, but little in the *Zostera*-meadows. Only *Ectocarpus confervoides, E. rufulus* and *E. siliculosus* have been recorded. Among the Chlorophyceae species of *Cladophora* are well represented. They prefer sea water with a low percentage of NaCl.

Many of the algae are epiphytic. Where the bottom of the sea is rocky there are usually well pronounced associations of various species. The environs of Heligoland may serve as an example. The less the rocks are resistant to wearing off by waves, the more quickly must the algae grow against the surface of the stones. *Ascophyllum nodosum* grows well on granite. Quick fastening species are *Enteromorpha lingulata, Alothrix flacca* and *Urospora pencilliformis* among the Chlorophyceae, *Bangia fuscopurpurea* among the Rhodophyceae and *Pylaiella litoralis* among the Phaeophyceae. Algae of the supra-littoral and littoral zones exhibit a more or less horizontal arrangement. *Fucus platycarpus* occurs, according to Nienburg (228, 229), in the upper part, at a depth of 1 to 2 meters. This species may be followed by *F. vesiculosus* at 1.10 to 0.40 meter. At 0.50 meter there is *F. serratus.* Species of *Laminaria* occur in the sublittoral zone, *e.g., L. digitata, L. saccharata* and *L. hyperborea,* of which *L. saccharata* grows to a depth of 1.10 meters and is followed by *L. digitata* and *L. hyperborea* to 4 meters where they are associated with several other species of Phaeophyceae and Rhodophyceae. At a depth of 5 to 10 meters there are in certain parts near Heligoland, *Valonia ovalis,* a species of Chlorophyceae, a few Phaeophyceae and 12 species of Rhodophyceae. On the muddy soils are *Enteromorpha, Chaetomorphe, Ulva, Ectocarpus, Ceramium* and *Ulothrix,* as well as a number of sulphur bacteria. Van Goor found that *Microcoleus chtonoplastus,* one of the Cyanophyceae, is able to keep mud particles together during high tide and can therefore be considered as the first that is able to hold the accumulated soil.

The first among land spermatophytes on mud flats is usually *Salicornia herbacea*, succeeded by *Halimus portulacoides* and sometimes by *H. pedunculatus*. Also *Scirpus maritimus* with its strong rhizomes is an excellent mud-binder. The coast along the southern half of the Zuider Zee (331) is lined in many parts by *Phragmites communis*. Along other places where the mud has settled are groups of *Glyceria maritima, G. distans* and *Juncus Gerardi*. On some salt meadows are extensive areas covered with *Glyceria maritima*. The *Salicornia*-zone may have as much as 0.21% NaCl, and the *Glyceria*-zone 0.03%; on such meadows *Hordeum maritimum* and *H. secalinum* occur not infrequently. Among the grasses are *Armeria maritima* and *A. elongata*, often associated with *Plantago maritima, Artemisia maritima, Glaux maritima, Cochlearia officinalis, C. anglica, C. danica, Erythraea pulchella, Statice Limonium, Triglochin maritimum, Bupleurum tenuissimum, Aster Tripolium, Obione portulacoides, Suaeda maritima, Atriplex hastatum* and *A. litorale*. Natural or artificial elevations that gradually come to lie outside the reach of salty water are inhabited by *Althaea, Malva, Heracleum, Pastenaca, Dactylis* and others having no connection with a typical halophilous life.

Generally, the flora along the North Sea and Baltic Sea is similar in construction, although there are a number of differences that must not be overlooked. An interesting member in this region is *Crambe maritima*, growing between rocks along the shores, and frequently subject to waves. Oliver (234) and Hill and Hanley (138) have made a study of the shingle beaches, where the sea is constantly throwing up shingle and sand, sometimes mixed with sand and stones. They found *Salicornia* and *Obione* where mud becomes thicker; the former, however, soon disappears, *Obione* being often in pure associations. Where the mud dies out *Obione* also disappears. Where the surface soil is principally sand, and sand and stones, *Suaeda fruticosa* is the characteristic plant. Along the shingle bank, vegetation is very sparse. Here the highest tides reach the topmost limit of the *Suaeda*-zone.

Mediterranean area. Arenes (10) studied the halophytic zones in various parts of Provence (France) along the Mediterranean. It is of interest to note that along the lower rocks near the coast there are *Statice minuta, Glaucium luteum, Juncus acutus, Crithmum maritimum, Polygonum Roberti* and *Samolus Valerandi*, re-

placed a little higher by *Crithmum maritimum, Artemisia gallica, Matthiola incana, Anthyllis Barba Jovis* and some other species, whereas at still higher elevations the halophytes are replaced by obligate glykophytes.

Along the mud-flat salt marshes are associations of *Salicornia fruticosa, S. macrostachys* and *S. radicans* which in many places are also the first colonizers. Where the land is less subject to tidal waves we find *Suaeda maritima* and *Aster Tripolium.* On dryer areas associations of *Linum maritimum* and *Dorycnium gracile* become considerably richer in species, among which may be mentioned *Statice serotina, Trifolium maritimum, Plantago Bellardi, Dactylis litoralis, Hordeum maritimum* and *Melilotus messanensis.* In the *Sonchus maritimus-* and *Plantago crassifolius*-association, *Statice virgata, Samolus Valerandi, Plantago coronopus, Erythraea spicata, Bellis annua, Pilicaria sicula* and some other species are to be found.

The vegetation of sandy shores is composed of the *Salsola*-association consisting of *Malcolmia parviflora, Frankenia pulverulenta, Allium Chamaemoly, Kocleria villosa, Cakile maritima, Matthiola sinuata, Frankenia laevis, Orlaya maritima, Bellis annua, Triglochin Barrelieri, Salsola Kali, S. Soda, Polygonum maritimum, Frankenia intermedia* and *Plantago arenaria.* Characteristic of the *Sporobolus pungens*-association are *Eryngium maritimum* and *Hutchinsia procumbens.*

In the salt marshes are associations of *Statice virgata* with *Obione portulacoides, Beta maritima, Inula crithmoides, Artemisia gallica* and *Lychnis laeta.* There are also associations of *Matthiola tricuspidata* with *Lagurus obvatus, Samolus Valerandi, Atriplex Tornabeni, Statice serotina, Salsola Kali, S. Soda, Sonchus maritimus* and others. In the association of *Suaeda splendens* there may be *Salicornia herbacea, Statice Girardiana, Triglochin maritimum* and *Kochia hirsuta,* and in the *Scirpus*-association, *S. pungens, Carex hispida, Orchis latifolia, Helosciadium nudiflorum* and *Galium palustre.*

Along the eastern half of the Mediterranean are a number of other interesting conditions. Ganiatsas (98, 99) made a study of the halophytes near Saloniki (Greece) and found that *Salicornia herbacea* occurs on places that contain the most salt, whereas *S. fruticosa* grows in locations with less salt. This association of *Sali-*

cornia fruticosa also harbors some plants of *Spergularia salina, Statice limonium, Cressa Cretica, Frankenia pulverulenta* and *Spheopsis divaricatus.* Where the salt content is still less in the soil one notices *Juncus acutis* with *Statice limonium, Plantago coronopus, Cynodon dactylon, Cressa cretica, Festuca rubra, Trifolium fragiferum* and *Tetragonolobus siliquosus.* From the other locations the following halophytes are described as accompanying *Salicornia herbacea: Salicornia radicans, Suaeda maritima, Spergularia salina, Chenopodium glaucum, Atriplex roseum, Salsola Kali, Hordeum maritimum, Dactylis litoralis* and *Festuca rubra,* occurring in various zonations. Salt meadows are covered mainly by *Cynodon dactylon, Festuca rubra* and *Trifolium fragiferum.* Further removed and where the salt content is less, the various associations become gradually richer in species of a glykophytic nature. Krause (179, 180) states that the halophytes of Asia Minor may be divided into two distinct groups, those growing directly along the coast and those of the inland salt deserts and salt lakes. Most of the species are fundamentally the same. The area along the coast is usually a narrow strip.

Along the north coast there are *Juncus acutis, Salsola Kali, Atriplex portulacoides, A. roseum, Polygonum maritimum, Cakile maritima, Medicago maritima, Euphorbia paralias, Diotis candidissima* and others.

Along the western and southern Mediterranean coasts are *Salicornia fruticosa, Aeluropus litoralis, Lotus halophilus, Frankenia pulverulenta, F. hirsuta* and *Orlaya maritima.* Along the Black Sea, especially prevalent, are *Scirpus maritimus, Pancratium maritimum, Crithmum maritimum, Suaeda maritima, Obione verrucifera, Eryngium maritimum, Statice Gmelini* and *Scabiosa maritima. Silene euxina* in the north and *S. discolor* in the southwest apparently have limited distributions.

Southern Asia. A number of salt lakes southeast of Calcutta have been studied by Kalipada. Their vegetation is in part composed of floating algae, or algae at the bottom in the mud. Kalipada distinguishes three zones or "stories" of vegetation. The first is composed of Cyanophyceae, *e.g., Oscillatoria princeps, O. limosa* and *O. laetevirens.* There may occur among the plankton a yellowish green film of *Euglena* with which are associated *Pandorina morum, Arthrospira platensis* and *Clathrocystis aeruginosa.*

Among the green filamentous algae are *Enteromorpha intestinalis, E. prolifera* and *Chaetomorphe Linum*. The second zone is composed of a number of spermatophytes growing in groups in the salt marshes, which include *Suaeda maritima, Heliotropium curassavicum* and several species of mangroves that will be mentioned later. In the shallower parts of the lakes *Phragmites Karka* may be noticed.

In his studies on the Indo-Malayan strand flora, excluding the mangrove, Schimper (273) distinguishes *Nipa*- and *Barringtonia*-formations which are composed of woody plants, and also a *pes-caprae*-formation which is composed mainly of herbaceous plants. Cruciferae and Caryophyllaceae, which often occur abundantly along the shores of the temperate zones, are conspicuously absent from the tropics; also the Chenopodiaceae and Tamariscaceae are not very numerous. The Indo-Malayan flora is represented by *Spinifex squarosus, Ischaemum muticum, Zoysia pungens,* among the grasses, and also by *Crinum asiaticum, Pancratium zeylandicum, Tacca pinnatifida, Euphorbia Atoto,* and by species of *Boerhaavia, Pandanus, Triumgetta, Crotalaria, Vigna, Canavalia* and *Aeschynomena; Ipomoea pes-caprae* is everywhere very common. Species of *Pandanus* occur frequently in typical formations; that of *P. labyrinthicus* reminds one of a mangrove association.

Australia. Collins (61), Diels (71) and others have furnished data about the vegetation along the coast of Australia. At many places there is a well developed mangrove. Here and there are salt marshes which Collins mentions as forming two associations near Sydney: (*a*) a salicornietum composed of *Salicornia australis* with *Samolus repens, Suaeda australis* and *Spergularia rubra;* and (*b*) a juncetum formed by *Juncus maritimum* with some patches of *Casuarina glauca.* In some places associes can be recognized, among which are *Salicornia-Suaeda*-associes.

New Zealand. An excellent resumé of the halophytic associations of New Zealand has been furnished by Cockayne (60). Below the low-tide mark, the large brown algae formation is composed mainly of large species forming a continuous covering below which is a growth of smaller species of Rhodophyceae and Phaeophyceae. Of importance are the *Durvillea*- and the *Carpophyllum*-associations. Several species of algae are found in each. From about half-tide mark to below low-water level is the *Zostera*-asso-

ciation of which *Z. nana* makes pure stands on places that are muddy to more or less sandy in the estuaries. Salt swamps are formed in the shallow estuaries by *Avicennia officinalis.* The *Salicornia*-association represented by *S. australis* forms dense masses on tidal mud flats from slightly above the high-water mark. This species is daily covered by tidal water during spring tides. It traps the mud and in time prepares the soil for other halophytes. They pave the way for an association almost entirely composed of *Juncus maritimus* var. *australiensis* and *Leptocarpus simplex,* but also including *Carex litorosa, Scirpus americanus, S. robustus* and *Plagianthus divaricatus.* This plant growth is found on those tidal rivers and estuaries which are exposed to the highest spring tides.

Among the minor communities on the salt meadows and along some of the sluggish streams are *Mimulus repens* and some *Triglochin striata* var. *filicifolia, Scirpus americanus* and *Cotula coronopifolia.* The communities submerged in brackish water are formed by *Ruppia maritima, Althenia bilocularis* and *Zannichellia palustris,* according to locality. From here we gradually approach the salt meadow. Its primitive members are *Leptocarpus simplex, Triglochin striatum* var. *filicifolium, Juncus maritimus* var. *australiensis, Chenopodium glaucum* var. *ambigium, Salicornia australis, Spergularia media, Apium prostratum, A. filiforme, Plagianthus divaricatus, Selliera radicans* and *Cotula coronopifolia.* There is, according to Cockayne, no regularity in abundance of any of the species. The salt meadow frequently succeeds the salt swamp, depending upon the rise of the soil surface above sea water and the influence of the tides. The same author mentions the coastal moor and related communities where a turf is formed from rhizomatous halophytes and certain coastal ferns and low-growing types of a subalpine-subantarctic appearance. Among the former are species of *Salicornia, Samolus, Selliera* and *Cotula,* and among the latter are *Blechnum durum, Asplenium obtusatum, Agrostis muscosa, Rumex neglectus, Montia fontana, Myosotis pygmaea* var. *Traillii, Euphrasia repens, Asperula perpusilla* and *Plantago Hamiltonii.* These moors are formed especially south of South Otago and the Stewart Districts, depending upon an acid peaty soil and a subantarctic climate. Due to showers of sea spray there is considerably more salt in the soil than can otherwise be expected in moist bogs. The last communities are those of the seashore and the

beach of loose stones. The former contains the upper strata of loose sand which is above the usual high-water mark, but unusual high tides subject it to sea water; here most land plants could not thrive, but some dune plants—*Spinifex hirsutus, Carex pumila, Desmoschoenus spiralis*—are encountered.

Common along the coast is a formation on beaches of loose stones, varying in its composition according to the fineness of the sand and the size of the stones. Among the large boulders are the most common halophytes, *e.g., Apium prostratum, Calystegia Soldanella* and *Senecio lautus;* more removed from the waves are *Phormium tenax, Scirpus nodosus, Mariscus ustulatus* and *Lobelia anceps.* Where the soil is finer, about 88 species have been found, composed of halophytes and non-coastal shrubby and herbaceous mat plants, among which are *Deyeuxia Bellardieri, Muehlenbeckia complexa, Mesembryanthemum australe, Tetragonia expansa, Ranunculus acaulis, Linum monogynum* and *Pimelia prostrata.*

MANGROVES

Along the coasts of most tropical and subtropical shores grows a type of vegetation composed of woody plants and known as a mangrove. Most mangroves are distinctly amphibious; usually they live in the mud, but some develop over coral reefs. Ecologically they form a separate group entirely different from other halophytes. In earlier centuries they drew attention and were thoroughly described by Reede tot Drakenstein (257) in 1685 and by Rumphius (268) in 1743.

The members of these formations belong to different families, but many have developed very much the same morphological characteristics that would adapt them to the same ecological requirements. As to their distribution two areas can be readily defined: (*a*) the eastern section which comprises the coast of East Africa to Asia, reaching into Micronesia; and (*b*) the western section which includes West Africa and the coast of America. The majority of species are in the eastern mangrove groups. Here we find among the Rhizophoraceae the genera *Rhizophora, Bruguiera* and *Ceriops;* in Verbenaceae, the genus *Avicennia;* in Combretaceae, *Lumnitzera;* in Soneratiaceae, *Sonneratia;* in Meliaceae, *Xylocarpus;* in Myrsinaceae, *Aegiceras;* in Rubiaceae, *Scyphiphora;* and in Acanthaceae, *Acanthus* (*A. ilicifolius*).

In the western mangrove region are but a few (4) species and among the genera are *Rhizophora, Laguncularia* (Combretaceae) and *Avicennia.*

Morphologically many mangroves show peculiar adaptations, especially as to roots, the anatomy of water-storing tissue in the leaves, and their vivipary. Species of *Rhizophora* are characterized by their enormous system of brace-roots, anchoring in the mud, which are visible mainly during low tide, upholding the crown of the tree above the normal high tide. *Avicennia* and *Sonneratia* form vertical roots above ground, pneumatophores, as well as normal horizontal roots in the mud. In *Cereops Candolleana* the main roots are like props, being thick, whereas those that are deeply buried in the mud are thin and slender. *Bruguiera caryophylloides* forms roots near the surface that have the appearance of knee-like structures which, according to Schimper (273), have the same role as pneumatophores.

A number of detailed studies have been made on the root systems of these plants. Troll (324) and Troll and Dragendorff (325) came to the conclusion that the aerial roots of *Sonneratia* lengthen and adapt themselves to the substratum in which they grow. The vertical roots form at certain depths below the mud surface more or less horizontal roots from which a new root system may arise above the old one which penetrates deeper into the soil due to the accumulation of mud. It has been shown that these roots give off CO_2, no doubt as a result of respiration. The knee-like aerotropic roots of *Bruguiera* that come close to the surface, later grow downward into the mud. Where the knees arise, a more or less dense system of lateral roots is produced. Goebel (105, 107, 109) attributed to the aerial roots of *Avicennia, Sonneratia, Bruguiera* and *Xylocarpus* the ability to supply the underground root system with oxygen by aid of a strongly developed aerenchymatic tissue in the cortex.

True vivipary is characteristic of a number of mangrove species. It is very prominent in *Rhizophora* due to the fact that the conspicuous large taproot hangs from the pendant fruits. After having reached a certain degree of maturity the young seedlings drop from the mother plant and fall straight into the mud where they then develop lateral roots. A well developed vivipary is known also in *Bruguiera* and *Cereops,* whereas in *Aegiceras* the seeds

germinate within the fruit. There is no vivipary in *Sonneratia* and *Avicennia.* These conditions have been studied by Karsten (156), Goebel (106, 107), Mullan (222) and others, and various stages have been well illustrated by Reede tot Drakenstein (257) and Rumphius (268).

Of special interest are formations of *Nipa fruticans,* a palm, which is often found over large areas toward the land side of mangrove swamps, especially in eastern Asia and Australia. There is a similar *Bractis* vegetation in some parts of South America.

In his classical work on the Indo-Malayan strand flora Schimper (273) mentions that the vegetation of the shores is the most distinctly outlined of all; 23 species are reviewed, among which are the palms *Phoenix paludosa* and *Nipa fruticans* in a special formation in the less salty lagunes in the neighborhood of the sea, reached only by the highest tides. He distinguishes, furthermore, the *Barringtonia*-formation, a forest strip beyond the sand zone on which are dying algae and sea-shells. This formation is composed of numerous woody plants among which *Barringtonia speciosa* is one of the dominants, accompanied by *Pandanus, Cycas circinalis, C. Rumphii, Casuarina equisetifolia, Cocos nucifera* and several others.

The different species composing mangroves form distinct zones along the coast of certain parts of northern Java; for instance, *Avicennia marina* is followed by the usually much taller *A. officinalis.* Where conditions are everywhere very much the same, zones are less distinct, as along some parts of the coast of southern Java near the island of Nosea, as was observed by Faber (274). Mullan (222) reports that in the salt-water lakes southeast of Calcutta, *Avicennia officinalis* settles on the outside and is followed by mixed growth of *A. officinalis* and *Aegiceras majus,* reaching a height of 10 to 20 feet, with *Sonneratia apetala* occurring here and there. Collins (61) gives us some information about the mangrove formations near Sydney, New South Wales.

Typical tidal marshes are first inhabited by *Avicennia officinalis* whose seeds drift up on the tides. They soon establish themselves, and, according to Collins, "are bound down by mats of the alga *Cladophora* until the first roots have made their appearance." *Avicennia* is found along the extreme limits of the formation, attaining 15 to 30 feet in height. In this tidal woodland there are also some

individuals of *Aegiceras majus* where the species reaches its southernmost limits. This species grows here mainly in the inner zone of the mangrove formation.

Harshberger (128), Uphof (322, 333) and others mention that along the coast of the southern half of Florida the shore is fringed with *Rhizophora mangle*. Some of the keys and smaller islands are encircled by this species and show no others in the formation. In a great number of other places *Rhizophora*, toward the land side, is associated with *Avicennia nitida*. In this association occur also *Laguncularia racemosa* and, further up, *Conocarpus erectus*. Here, also, is *Batis maritima*, a low shrub of common occurrence, as well as *Acrostichum aureum*, a fern. The composition of most parts of the West Indian Islands, Central America and of a part of tropical South America is fundamentally the same.

Von Luetzenburg (194) describes the coast of northern Brazil, and von Wettstein (353) mentions that along the coast, bays and lagunes of southern Brazil there are extensive formations of mangroves. They are composed mainly of *Rhizophora mangle* and *Avicennia tomentosa*. In the higher parts of these areas are *Hibiscus tiliaceus, Acrostichum lomarioides* and the white-flowering *Crinum attenuatum*. There are but few epiphytes, most occurring toward the land side, especially in those parts that merge into the ordinary forest, and here *Cattleya* and *Laelia* abound upon the branches.

The Galapagos Islands and islands of the South Pacific, otherwise known for their interesting endemic species, maintain the same mangroves as found elsewhere. Stewart (304), who studied many of these islands, found *Rhizophora mangle* everywhere fringing pools of brackish water. There are thickets of *Laguncularia racemosa* and of *Avicennia officinalis* with here and there dense growths of *Conocarpus erectus*. On higher grounds there often is *Hibiscus tiliaceus*.

In Malaya the seeds of the mangroves are dispersed during the monsoon, according to Mullan.

Whereas the most northern point of distribution of *Avicennia* mangroves in Africa, according to Ascherson (19), is near Safaga, Egypt, at 26° 40′ N. lat. and 27° 40′ E. long., Uphof (333) found the northern limit of this group along the Atlantic Ocean in North America to be between Ormond and Daytona, Florida, at about

30° N. lat. About 22 km. southward near Coronado Beach are the first individuals of *Laguncularia racemosa* and of *Rhizophora mangle;* the more southward the taller they grow. Along the Gulf of Mexico these two species are found as a few individuals near Tarpon Springs, and they become very numerous along the Ten Thousand Islands, whereas *Avicennia nitida* is known to occur on some of the coastal islands of Mississippi. *Rhizophora mangle* grows along the coast of Mexico, apparently as far as the States of Vera Cruz, Coachuila and Sinaloa.

Most mangrove species, though differing considerably from each other in anatomical structure, possess schizogenous lacunae in their inner cortex. The relatively soft cortex is strengthened by sclerenchymatic tissue, and the leaves are thicker than those of psammophilous salt plants. Mullan (221, 222) describes the anatomy of several species in great detail.

INLAND HALOPHYTES

Many inland areas, often far removed from the coast, support an extensive halophytic vegetation. Such floras are found prinicipally in salt lakes, salt marshes and salt prairies. Though many of the species are also along the coast, there are a number in these land areas that are strictly endemic.

As early as 1792 Cramer (64) drew attention to the fact that "Meerespflanzen" occur near Hohenenggelsen. These plants were again mentioned as late as 1912 by Brandes (38) from the same locality and as including, among others, *Samolus Valerandi, Glaux maritima, Spergularia salina, Aster Tripolium* and *Zannichelia palustris*. In the New World it was Pursh in 1807 who recorded certain halophytes from the salt springs near Onondago Lake, and Torrey (322) records a similar vegetation from certain parts along the shores of the Great Lakes, suggesting that "they constituted part of an early flora of those regions, when the lakes were filled with salt water." Later, Paine (248) noted the occurrence of plants of coastal origin in the interior of New York. Gradually, other localities came to the attention of botanists. Thus far the origin of the flora of some of these is hard to trace. Some consider them as relicts whereas Svenson (313) thinks it more likely that the seeds were distributed by birds and wind, or perhaps as a result of human agencies.

Associations of inland halophytes are frequently found in desert regions, as in certain parts of the Southwest of the United States, northern Libya, the Algerian Highlands between the Tell and the Sahara Atlas, in Mesopotamia, Turkestan, Persia, northern Chile, and elsewhere. It is also of interest to note that Kolkwitz (127, 128) and Schultz (282) mention some halophytes on NaCl-free soils in certain parts of central Germany where *Triglochin maritimum, Juncus Gerardi, Samolus Valerandi, Glaux maritima, Plantago maritima* and other species abound in the marl pits near Borxleben and other parts of the country. A number of examples will be given here of these various inland halophilous areas from various parts of the world.

Great Salt Lake. Great Salt Lake in northwestern Utah may serve as an example in the western part of North America. During the Pleistocene period it was a great body of fresh water with its highest level about 1000 feet above the present. It is 75 miles long and 38 miles wide. The water contains chiefly sodium chloride with smaller amounts of potassium, magnesium and calcium salts. According to the depth, the salt content varies from 13.8% at the surface to 27.72% at the bottom, and the lake, therefore, is one of the most saline on earth. Where the water attains a high concentration of salts, there are found only a few algae, *e.g.:* Myxophyceae: *Aphanothece utahensis,* near the surface for the most part; *Microcystis Packardii,* forming gelatinous layers; *Oscillatoria tenuis* var. *natans* and *O. tenuis* var. *tergestina;* Chlorophyceae: *Chlamydomonas* and *Tetraspora lubrica* var. *lacunosa.* The shore is generally barren. The *Salicornia*-zone, composed of *S. rubra* and *S. utahensis,* is the first of the halosere. The former species is endemic and forms dense caespitose colonies. *Allenrolfea occidentalis* comes second in toleration as a halophyte. A *Suaeda erecta*-zone is strongly pronounced at many places and is followed by *S. Moquinii. Distichlis spicata* is able to cover the entire area from the shore to beyond the zone of *S. Moquinii.* According to Flowers (87), *Atriplex hastata* never attains any degree of dominance. *Scirpus americana* and *S. palustris* occur in salt swamps and enter the area of the shore region where fresh water flows into the lake. The strand swamp may also be characterized by *Potamogeton pectinatus, Ruppia maritima, Polypogon monspeliensis* and *Puccinellia Nuttalliana.* The average salt content of the *Sali-*

cornia-zone is 2.5% in the Tooele Valley. This salt content differs according to the season, being higher in summer, due to evaporation. The fourth zone is formed by *Spartina gracilis, Eriocoma cuspidata, Puccinellia Nuttalliana, Bromus textorum, Abronia salsa* and *Sphaerostigma utahensis.* Among the members of the fifth zone the most noticeable are *Sporobolus asperifolius, Distichlis spicata, Atriplex hastata, A. rosea, A. confertifolia* and *Suaeda Moquinii.* The last two zones are the most variable in sociological composition, whereas the pioneer types from the first zone are the most constant. On mud flats *Salicornia rubra* is followed by *Allenrolfea,* and there are more or less distinguishable zones of *Atriplex hastata,* succeeded by *A. truncata* and then by a mixed association of *Hordeum jubatum, H. Gussonianum, Polygonum aviculare, Sarcobatus vermiculatus, Lepidium perfoliatum, L. pubicarpum, etc.,* whereas the seepage areas are occupied by *Puccinellia Nuttalliana, Hordeum jubatum, Atriplex hastata, Spergularia salina, Ranunculus eremogenes* and *Halerpestes cymbalaria.*

As far as muddy banks and meadows are concerned there are three main groups, according to the degree of halophytism. Where the salt content is strong we find *Puccinellia Nuttalliana, Distichlis spicata, Hordeum jubatum, H. Gussonianum, Sitanion, Suaeda erecta, Atriplex rosea, A. argentea, A. hastata, Triglochin maritimum, Sporobolus airoides, Aplopappus lanceolatus, Iva axillaris* and others. The playas which are numerous and, according to Flowers, alike in soil, alkali and water relations, are successively followed by *Salicornia rubra, S. utahensis, Suaeda erecta, Allenrolfea occidentalis, Distichlis spicata* and finally by *Sarcobatus vermiculatus.* These areas may be covered by a single species, thus giving rise to *Salicornia*-flats, *Allenrolfea*-flats, *Distichlis*-meadows or *Sporobolus*-fields.

The alkaline plains which cover higher areas than the playas and where the accumulation of water is less, have a different, often mixed, flora. Flowers distinguishes a number of communities, *e.g.,* one of *Sarcobatus vermiculatus* and *Atriplex confertifolia* which is most extensive and found in most saline regions of the Southwest. It forms the edaphic climax vegetation of the Great Salt Lake region and is invaded by *Artemisia tridentata,* especially at its limits. Further, there are communities of *Kochia vestita* and *K. americana,* of *Kochia-Suaeda-Sarcobatus-Grayia* and of *Chryso-*

thamnus, characteristic of the lowlands and thriving in mildly saline soils. From a successional aspect Flowers comes to the conclusion that there are two pioneer types of the halosere in that area, namely, *Salicornia* and *Allenrolfea. Salicornia* is usually succeeded by *Suaeda erecta,* and *Allenrolfea* by *Distichlis.* The two pioneers are usually not found near each other as colonizers of the barren flats but are often associated at some distance from the line of invasion. It is difficult to find a uniform line of *Distichlis, Sporobolus, Atriplex* and *Suaeda,* which invade such extreme and variable areas.

Salt-marsh plants of Kansas. Schaffner (271) describes the following picture of inland halophytes in the salt marshes of Kansas. Through the central part of that State salt marshes and salt springs are relatively common, the largest being Big Marsh. A considerable part of it is barren, except some isolated places where a few salt plants occur. Outside the barren area are seven zones of vegetation, especially well marked where the ground rises gently above the marsh. The first is but a few yards wide and entirely covered by *Distichlis maritima;* the second, a narrow strip, is formed by *D. spicata, Polygonum ramosissimum* and *Suaeda diffusa.* This zone is considered to be just above the limit of ordinary inundation by the brine, and is followed by a wide dry zone covered by a rather stunted growth of *Distichlis spicata* with *Polygonum ramosissimum* (rare), *Suaeda diffusa* (common) and *Iva ciliata* (rare). The fourth zone is composed of *Distichlis maritima* (characteristic), *Polygonum ramosissimum* (not very common), *Suaeda diffusa* (occasional), *Iva ciliata, Sporobolus heterolepus* (characteristic), *S. texana, Atriplex expansa* and *Aster multiflorus.* In the other zones several invaders become more and more dominant whereas the original three members disappear or decrease in size.

Salt spots along the Santa Cruz. Canon (48) has drawn attention to the plants in salt spots of the Santa Cruz by old Fort Yuma near Tucson, Arizona. He mentions especially *Atriplex canescens, A. polycarpa, A. elegans, A. Nuttallii* and *Suaeda suffrutescens.* These species occur in zones. He emphasizes the fact that in "alkali" areas, some parts are occupied mainly by *A. canescens,* others by *A. polycarpa* associated with species of *Lycium* and *Suaeda.* Near the center is *A. Nuttallii* whereas at the very center of the salt spot there is no vegetation at all. Chemical analysis

showed that there was 3.38% $MgSO_4$, 70.78% Na_2SO_4, 4.42% KCl and 15.48% $NaHCO_3$. These salts are not uniformly distributed and as a consequence give rise to different associations. Salts were most abundant in the *A. Nuttallii*-zone and least in the *A. canescens*-zone.

Halophytic areas in northern Chile. Salt marshes are not rare in some parts of the higher Andes. Much of these marshes is covered by *Oxychloë andina* (Juncaceae) where, according to Werdermann (352), there is no other species. Salt often occurs abundantly in crystallized form along the shores where it frequently covers parts of the plants above the ground. *Lycium humile,* strongly resistant to salt, is found in this region.

Reiche (258) mentions *Nitrophila occidentalis* and loose shrubberies of *Atriplex atacamense, Ephedra andina* and *Tessaria absinthoides* along the margins of the salt lakes of Atacama. From near Vegas de Carvajal he mentions salt meadows covered with *Distichlis* and here and there with *Nitrophila axillaris.* In other halophilous localities there is *Triglochin maritimum* var. *atacamensis,* often densely covered by loose particles of salt, and considered by Reiche as the most halophilous spermatophyte in this region.

Semi-deserts along the lower Volga. Keller (161, 164, 165) gives us an account of the large semi-desert regions from the lower Volga toward the foothills of the Altai Mountains, an area where the presence of NaCl and of Na_2SO_4 is especially characteristic. Near the Caspian Sea are enormous salt lakes, and about 700 of them are in the former province of Astrakan. Where considerable amounts of salt are found and a sufficient humidity during summer, one encounters *Atriplex verruciferum, Petrosimonia crassifolia* and *Halocnemum strobilaceum,* and on the more moist localities are associations of *Salicornia herbacea.* Not infrequently white salt crusts are to be seen on the surface of the soil. Where there is a considerable amount and depth of these salts together with drought during the hot summer, there are associations of *Kochia prostrata, Camphorosma monspeliaceum, Artemisia pauciflora* and *Nanophytum erniaceum.* Where there are transitions between the two groups there are often *Anabasis salsa* and *Atriplex canum.* In the cells of the plants in the first of these groups, there are, according to Keller, osmotic pressures up to 79 atmospheres. Some species appear to be distinctly succulent, as the perennial *Salicornia* and

Halocnemum and the annual *Petrosimonia* whose leaves are covered by hairs. Species of the dry salty soils, *e.g., Nanophytum, Camphorosma* and *Kochia,* are but slightly succulent. Where there is considerable underground water and less salt, there are large associations of *Lasiagrostis* (*Stipa*) *splendens.*

Inland halophytes of Asia Minor. According to Krause (179, 180), the halophytes along a narrow coastal strip of Asia Minor extend also to the inland salt marshes, salt deserts and salt prairies where there often is a higher temperature. Sometimes the salt content is so high that the soil can not support any vegetation, especially near the salt lake Tuz Čolü. In the salt region northeast of Knia he found *Aeluropus litoralis, Agropyrum prostratum, A. scirpeum, Scirpus lacustris, Juncus Gerardi* var. *condensatus, Panderia pilosa, Halianthium pilosum, Salsola Kali, Atriplex tataricum* var. *virgatum, Frankenia, Malcolmia africana* and *Statice globulifera.* In the salt steppe near lake Sultan Sasy, which was dried out during his visit and therefore had a high salt content, almost the same species were found, as well as *Kochia scoparia, K. prostrata, Statice pycnantha* and others. On weak alkali soils there are also a number of facultative halophytes, *e.g., Atropis distans, Convolvulus lineatus, Taraxacum farinosum, Alyssum desertorum* and *Androsace maxima.* They are small and dwarfed in saline soils where they produce fewer flowers and fruits, whereas in a glykophilous environment they are far more fertile and have a much more vigorous growth.

The Wadi Natrun in Egypt. Stocker (308), who made a study of the 120 km. long and 5 to 20 km. wide Wadi Natrun in Egypt, mentions the various plant associations resulting from the different salt contents of its waters and soils. The difference is often caused by evaporation giving different NaCl-concentrations, whereas the subsoil fresh water currents result in a dilution of the salt. There are formations of *Typha latifolia* that never bloom. Where the land becomes slightly higher *Typha* is replaced by *Eragrostis bipinnata.* At such places the salt content increases, due to capillarity and evaporation. In some parts also *Phragmites communis* var. *stenophila* abounds. Toward the higher edges of these *Eragrostis* marshes is often a zone of *Juncus acutis. Eragrostis* forces its rootstocks through the salt crusts near the surface into the subsoil containing less NaCl. The concentration at 40 cm. depth

is about 1.4% ; the upper surface is 4.8% at some places. In some regions of the Wadi Natrun, especially toward the south, the NaCl content is so high that plant growth is impossible; such parts are called Sebcha. Only toward the edges are there some sand heaps and small hills harboring *Zygophyllum album, Alhagi maurorum* and *Tamariscus.* Higher parts of the country contain less NaCl and are covered by a number of annuals and succulents, *e.g., Bassia muricata, Salsola inermis, Zygophyllum album, Nitraria retusa, Mesembryanthemum nudiflorum, M. Forskâlii, Cotula cinerea* and *Aizoon canariense.*

Smith and Hubbard (300) recently described some salt-loving grasses from South Africa. *Puccinellia augusta,* which may become a valuable forage grass on saline soils, was found on strongly saline areas in the Orange Free State and other parts of South Africa.

CULTIVATION OF HALOPHYTES

Halophytes are cultivated in most botanical gardens. As early as 1697 Blankaart (36) mentioned the cultivation of *Aster Tripolium* in gardens near Amsterdam. Hoffman (141) states that he grew *Plantago maritima* during a period of 15 years, from 1855 to 1870, in ordinary garden soil free of salt. Winkler (356) mentions how in the Hamburg Botanical Garden a number of mangrove species and other halophytes were grown with success where only fresh water was supplied; among these he mentions: *Acanthus ilicifolius, Aegiceras majus, Avicennia officinalis, Bruguiera eriopetala, B. gymnorrhiza, Nipa fruticans, Rhizophora conjugata, R. mangle* and *Sonneratia acida.* The plants were grown in pots and tubs. *Rhizophora mangle* and *Bruguiera eriopetala* regularly flowered. Species of mangroves are grown under glykophilous conditions also in the Botanical Garden of Buitenzorg, Java.

SUMMARY

Halophytes are plants characteristic of habitats which contain a higher percentage of salts than other plants, so-called glycophytes, are able to endure. The nature of halophytes can be explained only by considering the physiological resistance of the protoplasm toward solutions containing NaCl or other salts. This resistance is considerable among most marine algae and certain land spermatophytes, as *Salicornia, Batis maritima,* and many mangrove

species. The high osmotic pressure resulting from this physiological resistance is very pronounced, and in *Atriplex latifolia* it ranges from 32.1 to 34.6 atm. and in *Lumnitzera racemosa* it has been found as high as 41.93 atm.

The morphological differences among halophytes are manifested in different ways, as in the succulency of *Batis maritima* and of species of *Salicornia*. From this xerophytic appearance it was concluded by early investigators that transpiration in halophytes is similar to that of plants adapted to genuine xerophytic habitats, but it has since been shown that transpiration of halophytes is considerable. Genetical and cytological aspect of halophytes may some day yield many interesting data, especially when one bears in mind the view of Hagerup that when the plants are grown under unfavorable conditions, there may originate a high percentage of polyploid species.

Halophytes are found in all four major divisions of the plant kingdom, those among thallophytes being the most numerous, those of the pteridophytes the least so. On account of their symbiotic significance, mycorrhiza and halophilous lichens deserve more attention than they have heretofore received. Also the matter of dominance in the open sea needs careful investigation.

The number of species among halophytes is smaller than that of other plant associations. They are more numerous toward the tropics and become less in number as one approaches colder regions.

Zonation caused by a gradient in salt contents of the soil is displayed by several species of land plants, but particularly by halophytes. The zonation may be caused also by endurance of salt spray or high tide, as by *Salicornia* and certain lichens. Finally, it may be noted that ease of seed dissemination by the sea has given most species of halophytes considerable distribution over the earth and that this distribution of halophytes as a group is limited by temperature factors.

Typical halophytes are *Salsola Kali, S. Soda, Polygonum maritimum, Frankenia intermedia,* and *Plantago arenaria.* They are followed in toleration of salt, according to Arenes, by *Cakile maritima, Matthiola sinuata, Frankenia laevis, Orlaya maritima, Bellis annua* and *Triglochin Barrelieri,* and are then succeeded by a sandy shore vegetation of the *Salsola*-association among which there may

be *Malcolmia parviflora, Frankenia pulverulenta, Allium Chamaemoly* and *Koeleria villosa.*

BIBLIOGRAPHY

1. ADRIANI, M. J. Recherches sur la synécologie de quelques associations halophytes méditerranéennes. Stat. Int. Geobot. Med. et Alp. Montpellier. 1934.
2. ————. Sur la transpiration de quelques halophytes cultivées dans les milieux différents en comparaison avec celle de quelques non-halophytes. Proc. Akad. Wet. Amsterdam **40**: 524–529. 1937.
3. ALLEN, W. E. Problems of floral dominance in the open sea. Ecology **2**: 26–31. 1921.
4. ANAND, P. L. An ecological study of the algae of the British Chalk-cliffs. Jour. Ecol. **25**: 153–188. 1937.
5. ANDERSON, KNUD H. En salt-flora i slagsmose ved Rislev. Bot. Tidskr. **38**: 57–68. 1923.
6. ANDERSON, SVEND. Nye fund af halofyter i storebaeltsomraadets inland. Bot. Tidskr. **41**: 100–112. 1930.
7. ARBER, E. B. A. On the effect of salt on the assimilation of carbon dioxide in *Ulva latissima.* Ann. Bot. **15**: 39–69. 1901.
8. ————. On the effect of nitrates on the carbon-assimilation of marine algae. Ann. Bot. **15**: 669–681. 1901.
9. ARENDS, J. Ueber den Einfluss chemischer Agenzien auf Stärkegehalt und osmotischen Wert der Spaltöffnungsschliesszellen. Planta **1**: 84–115. 1925.
10. ARÈNES, J. Étude sur la zone halophyte en Provence. Bull. Soc. Bot. France **70**: 238–247. 1923; **71**: 93–117. 1924.
11. ARESCHOUG, F. W. C. Untersuchungen über den Blattbau der Mangroven-Pflanzen. Bib. Bot. Heft 56. 1902.
12. ————. Zur Frage der Salzabscheidung der Mangrovenpflanzen. Flora **93**: 155–196. 1904.
13. ARNOLD, AUGUST. Beiträge zur ökologischen und chemischen Analyse des Halophyten-problems. Jahrb. Wiss. Bot. **83**: 105–132. 1936.
14. ARNOTT, G. W. On the Rhizophorae. Ann. Nat. Hist. **1**: 359–367. 1838.
15. ARRHENIUS, O. Ökologische Studien in den stockholmer Schären. 1920.
16. ————. The hydrogen-ion concentration of Egyptian soils and the reclamation of alcaline land. Cairo Sci. Jour. **10**: 3–21. 1921.
16. ASCHERSON, P. Vorarbeiten zu einer Übersicht der phamerogamen Meeresgewächse. Linnaea **35**: 152–208. 1867.
17. ————. Mittheilungen über die phanerogamen Gewächse des Rothen Meeres. Sitzungsber. Ges. Nat. Freunde, Berlin **1870**: 83–85. 1871.
18. ————. Die geographische Verbreitung der Seegrässer. Petermanns Geogr. Mitt. **17**: 241–248. 1871.
19. ————. Der nordlichste Fundort der Mangroven in Egypten. Bot. Ztg. **61** Abt. (2): 235–238. 1903.
20. BACHMANN, F. Über die Beziehungen zwischen den Wassergehalte des Bodens und seinem Wasserdampfdrucke, sowie über diejenigen zwischen der Saugkraft des Bodens und dem Welken der Pflanzen. Planta **4**: 140–180. 1927.
21. BATALIN, A. Kultur der Salzpflanzen. Gartenflora **28**: 68–72. 1876.
22. ————. Wirking des Chlornatriums auf die Entwicklung von *Salicornia herbacea* L. Bull. Congr. Int. Bot. et Hort. St. Petersburg. 1886.

23. BATTANDIER, J. A. Quelques notes sur les causes de la localisation des espèces d'une région. Bull. Soc. Bot. France 34: 189–195. 1887.
24. BENECKE, W. Ueber die Dielsche Lehre von den Entchlorung der Halophyten. Jahrb. Wiss. Bot. 36: 179–196. 1901.
25. ———, UND A. ARNOLD. Zur Biologie der Strand- und Dünenflora. Ber. Deut. Bot. Ges. 49: 363–381. 1931.
26. ———, UND L. JOST. Pflanzenphysiologie. 4 Aufl. 1924.
27. BENJAMIN, M. S. Saltbush. Its mineral composition. Agr. Gaz. New South Wales 46: 309–310. 1935.
28. BERGER- LANDEFELDT, ULRICH. Die Hydratur einiger Halophyten in ihrer Abhängigkeit von der Substratkonzentration. Beih. Bot. Centralb. Abt. I 51: 697–710. 1933.
29. BERNATSKY, J. Ueber die Halophytenvegetation des Sodabodens im ungarischen Tiefland. Ann. Mus. Nat. Hung. 3: 62–72. 1905.
30. BERNINGER, E. Extreme Ausbildung einer Nebenvegetation in der nord-chilenische Wüste. Zeits. Ges. Erdk. Berlin. 1925.
31. BERTHOLD, G. Die Verteilung der Algen im Golf von Neapel. Mitt. Zool. Stat. Neapel 3: 431–464. 1882.
32. BERGER, S. Ueber den Einfluss des Meereswassers auf die Keimfähigkeit der Samen. Beih. Bot. Centralbl. 21: 262–270. 1907.
33. BICKENBACH, K. Zur Anatomie und Physiologie einiger Strand- und Dünenpflanzen. Beitr. Biol. Pflanzen (Cohn) 19: 334–370. 1932.
34. BIDWELL, G. L. Saltbushes and their allies in the United States. U. S. Dept. Agr. Bull. 1345. 1925.
35. BISWAS, KALIPADA. Flora of the saltmarshes. Jour. Dept. Sci. Univ. Calcutta 8(7): 1–47. 1927.
36. BLANKAART, STEPH. De Nederlandschen Herbarius of Kruidboek. 1697.
37. BLATTER, E. Mangrove of the Bombay Residency and its biology. Jour. Bombay Nat. Hist. Soc. 16: 644–656. 1905.
38. BRANDES. H. Ueber einen verlorengegangen Standort von Salzpflanzen. Jahresb. Niedersächs. Bot. Ver. Hanover. 1912.
39. BRAUN-BLANQUET, J. Pflanzensoziologie. 1928.
40. ———. Zur Frage der physiologischen Trockenkeit der Salzböden. Ber. Schweiz. Bot. Ges. 40: 33–39. 1931.
41. BRENNER, W. Strandzoner i Nylands skärgård. Bot. Not. 173–192. 1916.
42. BRICK, C. Beitrage zur Biologie und vergleichende Anatomie der baltischen Strandpflanzen. Schrift Nat. Ges. Danzig 7: 108–120. 1888.
43. BUDDE, HERMANN. Versuche ueber die Anpassung einiger Algen an den wechselnden Salzgehalt. Ber. Deut. Bot. Ges. 50: 343–349. 1932.
44. BULLOCK, DOLORES M. *Atriplex semibaccata* as influenced by certain environmental conditions. Ecology 17: 262–269. 1936.
45. BUROLLET. P. A. Considérations dynamogénétiques sur le "Salicornietum" de quelques sebkas Tunisiennes. Compt. Rend. Assoc. France Adv. Sci. 49: 352–355. 1926.
46. CANDOLLE, A. DE. Géographie Botanique Raisonée. 1855.
47. CANNON, W. A. On the electric resistance of solutions of salt plants and solutions of alkali soils. Plant World 11: 10–14. 1908.
48. ———. Some relations between salt-plants and salt-spots. Mem. Stanford Univ. 1913.
49. CARTER, NELLIE. A comparative study of the algae flora of two salt marshes. Jour. Ecol. 20: 341–370. 1932; 21: 128–208, 285–403. 1933.
50. CAVARA, F. Risultati di una serie di richerche crioscopiche sui vegetali. Contrib. Biol. Veget. 4: 41–47. 1905.

51. Chapman, V. The halophyte problem in the light of recent investigations. Quart. Rev. Biol. 11: 209–220. 1936.
52. ———. Studies in salt-marsh ecology. Jour. Ecol. **26**: 144–179. 1938; **27**: 160–201. 1939.
53. Chermezon, H. Recherches anatomiques sur les plantes littorales. Ann. Sci. Nat. Bot. IX **12**: 117–125. 1910.
54. Chouard, Pierre. Les tourbières de pelouses, ou pozzines dans les Pyrénées, formations homologues des pozzines de Corse. Bull. Soc. Bot. France **82**: 632–642. 1936.
55. Christ, H. Die Géographie der Farne. 1910.
56. Christiansen, W. Die Aussendeichvegetation von Schleswig-Holstein mit besonderer Berücksichtigung von Föhr. Föhrer Heimatsbücher nr. 16 Wijk. 1927.
57. ———Das pflanzengeographische und soziologische Verhalten der Salzpflanzen mit besonderer Berücksichtigung von Schleswig-Holstein. Beitr. Biol. Pflanzen (Cohn) **22**: 139–154. 1934.
58. Clements, F. E. Plant Succession: an analysis of the development of vegetation. Carnegie Inst. Washington Publ. 1916.
59. ———. Plant Indicators. Carnegie Inst. Washington Publ. 1920.
60. Cockayne, L. The Vegetation of New Zealand. 1928.
61. Collins, M. S. On the mangrove and salt-marsh vegetation near Sydney with special reference to Cabbage Tree Creek, Port Hacking. Proc. Linn. Soc. New South Wales **46**: 376–397. 1921.
62. Contejean, C. De l'influence du terrain sur la végétation. Ann. Sci. Nat. Bot. V **20**; VI **2**.: 1874–1875.
63. Cooper, R. E., and Pasha, S. A. The osmotic and suction pressures of some species of the mangrove vegetation. Jour. Indian Bot. Soc. **14**: 109–120. 1935.
64. Cramer, J. A. Physische Briefe ueber Hildesheim und dessen Gegend. 1792.
65. Daines, L. L. On the flora of Great Salt Lake. Am. Nat. **50**: 499–506. 1917.
66. Davis, Charles A. Salt marsh formation near Boston and its geological significance. Econ. Geol. **5**: 623–639. 1924.
67. Davis, W. C. Tidal-flat and salt-marsh studies in Nelson Haven. New Zealand Jour. Sci. & Techn. **12**: 338–360. 1931.
68. Delf, E. M. Transpiration and behaviour of stomata in halophytes. Ann. Bot. **26**: 409–415. 1911.
69. Denny, F. E. Field method for determining the saltness of brackish water. Ecology **8**: 106–112. 1927.
70. Diels, L. Stoffwechsel und Struktur der Halophyten. Jahrb. Wiss. Bot. **32**: 309–326. 1898.
71. ———. Die Pflanzenwelt von West-Australien. 1906.
72. Dobbs, C. G. The vegetation of Cape Napier, Spitzbergen. Jour. Ecol. **27**: 126–148. 1939.
73. Dodoens, Rembert. Crüydeboeck. 1563.
74. Drews, J. Regulation des osmotischen Druckes in Meeresalgen bei Schwankungen des Saltzgehaltes im Aussenmedium. Arch. Freunde Nat. Mecklenburgs Diss. 1896.
75. Dudby, W. R. *Phyllospadix* its systematic characters and distribution. Zoe **4**: 381–385. 1894.
76. Du Rietz, E. G. Gotländische Vegetationsstudien. Svenska Växtsoc. Sellkapets Handl. II Uppsala 1925.
77. Duval-Jouve, M. Observations anatomiques et morphologiques. Bull. Soc. Bot. France **15**: 132–140. 1868.
78. Eaton, F. M. The water requirements and cell-sap concentration of Australian saltbush and wheat as related to the salinity of the soil. Am. Jour. Bot. **14**: 214–226. 1927.

79. FABER, F. C. VON. Über die Transpiration und den osmotischen Druck bei den Mangroven. Ber. Deut. Bot. Ges. 31: 277–286. 1913.
80. ———. Zur Physiologie der Mangrove. Ber. Deut. Bot. Ges. 41: 227–234. 1923.
81. ———. Untersuchungen über die Physiologie der javanischen Solfatarenpflanzen. Flora 118/119: 89–98. 1925.
82. FEHER, D., UND VAGI, ST. Untersuchungen über die Einwirkung von Na_2CO_3 auf Keimung und Wachstum der Pflanzen. Biochem. Zeits. 158: 375–378. 1925; 159: 172–174. 1926.
83. FIORI, A. Le piante alofite e la liane. Al. Univ. For. Ital. 19: 464–466. 1932.
84. FITTING, HANS. Die Wasserversorchung und die osmotischen Druckverhältnisse der Wüstenpflanze. Zeits. Bot. 3: 209–275. 1911.
85. ———. Aufgaben und Ziele einer vergleichenden Physiologie auf geographischen Grundlage. 1922.
86. ———. Die ökologische Morphologie der Pflanzen im Lichte der neueren physiologischen und pflanzenphysiologischen Forschungen. 1926.
87. FLOWERS, S. Vegetation of the Great Salt Lake Region. Bot. Gaz. 95: 353–418. 1934.
88. FOCKE, W. O. Kulturversuche mit Pflanzen der Inseln und Küste. Abh. Naturw. Ver. Bremen. 4: 278–292. 1875.
89. FONSECA, O. O. R. DE. Vegetação e aspecto phytogeographico do Brazil. 1923.
90. FRÖDE, E. Die Halophytenfloren in Braunshweigs Umgebung. Jahresb. Ver. Naturw. 22: 35–45. 1933.
91. FROMAGEOT, C. Sur les relations entre l'état physico-chémique et le fonctionement du protoplasma. Phytosynthèse et respiration. Bull. Soc. Chim. Biol. 6: 169–180. 1924.
92. FUKUDA, YASONA. Die Anpassungsfähigkeit der Pflanzen bezüglich des osmotischen Druckes. Bot. Mag. Tokyo 51: 445–456. 1937.
93. GABRIELSON, E. K. Über den Kohlenstoffhaushalt der terrestrischen Halophyten. 1935.
94. GALLOE, O. Dansk Likeners Økologie. Dansk Bot. Tidskr. 28: 285–370. 1908.
95. ———. Forberedende Undersøgelser til en almindelig Likenølogie. Dansk Bot. Airk. 1 nr. 3. 1913.
96. GAMS, H. [In *Fr. Verdoorn:* Manual of Bryology. 1932.]
97. ———. [In *Fr. Verdoorn:* Manual of Pteridophytes. 1938.]
98. GANIATSAS, CONSTANTIN. Die Pflanzenassoziationen auf den Salzböden. Jahresb.Nat. Fak. Univ. Saloniki 3: 14–23. 1935.
99. ———. Untersuchungen über die Vegetation auf den Salzboden bei Saloniki. Ber. Deut. Bot. Ges. 54: 430–444. 1936.
100. GANONG, W. F. On halophytic colonies in the interior of New Brunswick. Bull. Nat. Hist. Soc. New Brunswick nr. 16: 50–52. 1898.
101. ———. Vegetation of the Bay of Fundy Marshes. Bot. Gaz. 36: 165–186, 280–302, 349–367, 429–555. 1903.
102. GESSNER, J. Die osmotischen Druckverhältnisse der Dünen- und Strandpflanzen der Nordsee bei verschiedener Salzkonzentration. Diss. Freiburg. 1920.
103. GILTAY, E. Anatomische Eigentümlichkeiten in Beziehung auf klimatische Umstände. Nederl. Kruidk. Arch. II. 4: 413–440. 1886.
104. GINZBERGER, A. Der Einflüss des Meereswassers auf die Gliederung der süddalmatischen Küstenvegetation. Oest. Bot. Zeit. 74: 114. 1925.
105. GOEBEL, K. Ueber die Rhizophoren-vegetation. Sitz. ber. d. Naturf. Ges. Rostock. Dec. 1886.
106. ———. Ueber die Luftwurzeln von *Sonneratia*. Ber. Deut. Bot. Ges. 4: 249–255. 1886.

107. ———. Pflanzenbiologische Schilderungen. 1889.
108. ———. Organographie der Pflanzen. 3: 3 Aufl. 1933.
109. Gola, G. Studi sui rapporti de la distribuzione della piante e la costituzione fisico-chimica del suolo. Ann. di Bot. 3: 455–512. 1905.
110. ———. Saggio di una teoria osmotica dell' edafismo. Ann. di Bot. 3: 275–360. 1910.
111. Goor, A. C. J. van. Die Zostera-Assoziation des holländischen Wattenmeeres. Rec. Trav. Bot. Nèerl. 18: 103–121. 1921.
112. ———. De halophyten en de submerse phanerogamen in: *Redeke*. Flora en Fauna der Zuiderzee. 47–53. 1922.
113. Gruner, H. Die Marschbildungen an den deutschen Nordseeküsten. 1913.
114. Halket, A. C. Effects of salt on the growth of *Salicornia*. Ann. Bot. 29: 143–155. 1915.
115. ———. Some experiments on absorption by the aerial parts of certain salt-marsh plants. New Phytol. 10: 121–139. 1911.
116. ———. The effect of salt on the growth of *Salicornia*. Ann. Bot. 29: 143–154. 1915.
117. ———. The morphology of *Salicornia*—an abnormal plant. Ann. Bot. 42: 525–530. 1928.
118. Hamilton, A. A. An ecological study of the saltmarsh vegetation in the Port Jackson District. Proc. Linn. Soc. New South Wales 54: 463–513. 1919.
119. Harder, R. Beiträge zur Kenntnis der Gaswechsels der Meeresalgen. Jahrb. Wiss. Bot. 56: 254–299. 1915.
120. ———. Ueber die Wasser- und Salzgehalt und die Saugkraft einiger Wüstenboden Beni Unifs. Jahrb. Wiss. Bot. 72: 566–699. 1930.
121. Harris, J. A., Gorter, R. A., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T. The osmotic concentration, specific electrical conductivity and chloride content of tissue fluids of the indicator plants of Tooele Valley, Utah. Jour. Agri. Res. 27: 893–924. 1924.
122. Harshberger, J. W. An ecological study of the New Jersey strandflora. Proc. Acad. Sci. Philadelphia. 623–671. 1900.
123. ———. Additional observations on the strandflora of New Jersey. Proc. Acad. Sci. Philadelphia. 642–669. 1902/03.
124. ———. Notes on the strandflora of Great Inagua, Haiti and Jamaica. Torreya 3: 67–70. 1903.
125. ———. The comparative leaf structure of the sanddune plants of Bermuda. Proc. Am. Phil. Soc. 47: 97–101. 1908.
126. ———. The comparative leaf structure of strand plants of New Jersey. Proc. Am. Phil. Soc. 48: 72–81. 1909.
127. ———. The vegetation of saltmarshes and of the salt and fresh water ponds of northern coastal New Jersey. Proc. Acad. Sci. Philadelphia. 373–400. 1909.
128. ———. The vegetation of south Florida, south of 27° 30′ North, exclusive of the Florida Keys. Trans. Wagner Free Inst. Sci. Phila. 7: 51–189. 1914.
129. ———. The origin and the vegetation of saltmarsh pools. Proc. Am. Phil. Soc. 481–484. 1916.
130. Hauman, L. Esquisse phytogéographique de l'Argentine subtropicale et de ses relations avec la géobotanique sud-américaine. 1932.
131. Hay, V. Marine algae of New Brunswick. Proc. Royal Soc. Canada 5: 167–174. 1887.
132. Häyrén, E. Ueber die Landvegetation und Flora der Meeresfelsen von Tvärminne. Acta Soc. Fauna et Flora Fennicae 39. nr. 1. 1914.
133. Hedley, C. An ecological sketch of the Sydney Beaches. Proc. Roy. Soc. New South Wales 59: 44–46. 1915.
134. Hewat, W. G. Ecological studies on selected marine intertidal commu-

nities of Monterey Bay, California. Am. Midl. Nat. 18: 161–206. 1937.
135. HILL, A. F. The vegetation of the Penobscot Bay Region, Maine. Proc. Portland Soc. Nat. Hist. 3: 308–318. 1923.
136. ————. Observations in the osmotic properties of the root hairs of certain salt-marsh plants. New Phyt. 7: 133–142. 1908.
137. ————. The water economy of maritime plants. Science Progress 14: 60–72. 1919.
138. HILL, T. G., AND HANLEY, J. A. The structure and water-content of shingle beaches. Jour. Ecol. 2: 21–38. 1914.
139. HOFFMAN, CURT. Zur Bestimmung des osmotischen Druckes an Meeresalgen. Planta 16: 413–432. 1932.
140. HOFFMANN, H. Ueber Kalk- und Salzpflanzen. Landw. Vers. Stat. 13: 269–304. 1871.
141. ————. Kulturversuche. Bot. Ztg. 35: 293–295. 1877.
142. HOLTERMANN, C. Anatomisch-physiologische Untersuchungen in den Tropen. Sitzungsber. Preuss. Akad. Wiss. Berlin. 1902.
143. ————. Der Einfluss des Klimas auf den Bau der Pflanzengewebe. 1907.
144. HOLTTUM, R. E. [In *Fr. Verdoorn.* Manual of Pteridology. 1938.]
145. HUBER, B. Die Beurteilung des Wasserhaushaltes der Pflanzen. Jahrb. Wiss. Bot. 64: 1–120. 1924.
146. ILJIN, W. S. Physiologischer Pflanzenschutz gegen schädliche Wirkung von Salzen. Biochem. Zeits. 132: 526–540. 1922.
147. ————. Ueber verschiedene Salzbeständigkeit der Pflanzen Sitzungsber. K. Böhm. Ges. Wiss. Kl. II. 1923.
148. ————. Synthesis of starch in plants in the presence of calcium and sodium salts. Jour. Ecol. 6: 333–351. 1925.
149. ————. Anpassung der Halophyten an konzentrierte Salzlösungen. Planta 16: 352–366. 1932.
150. IRVING, W. The salts of the Dead Sea and River Pordan. Geogr. Jour. 61: 428–440. 1923.
151. ISAACK, W. E. Preliminary study of the water loss of *Laminaria digitatata* during intertidal exposure. Ann. Bot. 49: 109–117. 1935.
152. JOHNSON, DUNCAN S., and SKUTCH, ALEXANDER F. Littoral vegetation on a headland of Mt. Desert Island, Maine. Ecology 9: 188–215. 1928.
153. ————, AND H. H. YORK. The relation of plants to Tidelevels. Carnegie Inst. Washington Publ. 1915.
154. KAHO, H. Das Verhalten der Pflanzenzelle gegen Salze. Ergebn. Biologie. 1: 380. 1926.
155. KAMMERLING, Z. Welche Pflanzen sollen wir "Xerophyten" nennen? Flora 106: 433–460. 1904.
156. KARSTEN, G. Ueber die Mangrovenvegetation im Malayischen Archipel. Bib. Bot. Heft 17. 1891.
157. KEARNEY, T. H. Are plants of sea beaches and dunes true halophytes? Bot. Gaz. 37: 424–436. 1904.
158. ————. Plant life on saline soils. Jour. Wash. Acad. Sci. 8: 109–125. 1918.
159. KEARNEY, T. H., BRIGGS, L. J., AND SHANTZ, H. C. Indicator significance of vegetation in Tooele Valley, Utah. Jour. Agr. Res. 1: 365–418. 1914.
160. KELLER, B. Quelques données sur la force osmotique du sac cellulaire des plantes en rapport avec le caractère des habitats. Mém. Inst. Agron. l'Emp. Pièrre a Voronèje 2: 1–23. 1916.
161. ————. Die Pflanzenwelt der russische Steppen, Halbwüsten und Wüsten. Woronech. 1923.

162. ————. Die Vegetation von Russland in Bilder. Lief. 1 Steppen, Halbwüsten und Wüsten. 1923.
163. ————. Halophyten und Xerophytenstudien. Jour. Ecol. **13**: 224–261. 1925.
164. ————. Die Vegetation auf den Salzboden der russischen Halbwüsten und Wüsten. Zeits. Bot. **18**: 113–137. 1926.
165. ————. Die Vegetation der Salzboden in der grossen Halbwüste des Bundes U. S. S. R. Vegetationsbilder 18 Heft 8. 1928.
166. ————. Die Einsalzung, die Transpiration und Salzanhäufung bei *Atriplex verruciferum.* Trudy Bot. Opytn. Stant. Voronezh. **1**: 75–78. 1929.
167. ————. Die Pflanzen in ihrem Verhältnisse zur Einsalzung. Trudy Bot. Opytn. Stant. Voronezh. **1**: 91–94. 1929.
168. Killian, Charles. Etudes écologiques sur la répartition du chlorure de sodium dans les psammophytes et halophytes algériens. Ann. Phys. & Physochim. Biol. **7**: 419–469. 1932; **11**: 70–124. 1935.
169. Kjellmann, F. R. Ueber die Algenvegetation des Murmanscher Meeres. Nova Acta Reg. Soc. Sci. Upsala. Ser. III. 1877.
170. ————. Ueber die Algenregionen und Algenformationen im östlichen Skagerrak. Bihang Svensk Vet. Akad. Handl. 5 nr. 6. 1878.
171. Klecka, A., a V. Vukolov. Srovnávaci studie o mykorrhize lučnich halofytů. (Vergleichendes Studium ueber Mykorrhiza der Wiesen Halophyten) Sbornik Ceskoslov. Akad. Zeměd. **12**: 190–195. 1937.
172. Kniep, Hans. Beiträge zur Keimungsphysiologie und Biologie von *Ficus.* Jahrb. Wiss. Bot. **44**: 635–640. 1907.
173. ————. Ueber die Assimilation und Atmung der Meeresalgen. Int. Rev. Ges. Hydrobiol. & Hydrogr. 1914.
174. Knight, J. A salt-marsh study. Am. Jour. Sci. **28**: 161–181. 1934.
175. Kolbe, R. W. Zur Okologie, Morphologie und Systematik der Brakwasserdiatomeen. Die Kieselalgen der Sperenberger Salzgebietes. Kolkwitz Pflanzenforsch. Heft 7. 1914.
176. Kolkwitz, R. Bericht d. kgl. Landesanstalt für Wasserhygiene über Untersuchungen im Bereiche des Flutkanals der Unstrich in der Zeit vom 3 bis 8 Juli 1915. Mitt. Kgl. Landesant. Wasserhyg. Heft. 22. 1917.
177. ————. Ueber die Standorte der Salzpflanzen. Ber. Deut. Bpt. Ges. **35**: 518–526. 1917.
178. ————. Ueber die Standorte der Salzpflanze. Ber. Deut. Bot. Ges. **36**: 636–645. 1919.
179. Krause, K. Salzpflanzen in Kleinasien. Naturwiss. **18**: 546–549. 1930.
180. ————. Ueber die Vegetationsverhältnisse des nordostlichen Kleinasien. Bot. Jahrb. **65**: 349–379. 1933.
181. Kylin, H. Ueber die Kälteresistenz der Meeresalgen. Ber. Deut. Bot. Ges. **35**: 370–374. 1917.
182. ————. Svenska västkustens algregioner. Svensk Bot. Tidskr. **12**: 65–90. 1918.
183. Lagatu, H., et H. Sicard. Contribution à l'étude des terres salées du Littoral Mediterranéen. Ann. d. Epiph. Ann. Ministère Agr. France. 1911.
184. Langendonck, H. J. van. De vegetatie en ocologie der schorrenplanten van Saaftingen. Een bijdrage tot de studie der halophyten. Bot. Jaarb. **23**: 1–128. 1932.
185. ————. La sociologie végétale des schorres du Zwijn et de Philippine. Bull. Soc. Roy. Bot. Belge **65** (II): 112–136. 1933.
186. Lans, R. Die Halophytenflora des südlichen Mähren und ihre Beziehungen zur Flora der Nachbargebiete. Mitt. Komm. Nat. Durchf. Mährens. Bot. Abt. nr. 3. 1901.

187. LEEUW, W. C. DE Halophtenonderzoek Eemgebied. Ned. Kruidk. Arch. 81–93. 1929.
188. LESAGE, P. Recherches expérimentales sur les modifications des feuilles chez les plantes maritimes. Rev. Gén. Bot. 2: 55–65, 106–109, 163–170. 1890.
189. LINDEMAN, C. A. M. A vegetação no Rio Grande do Sul. 1926.
190. LINNAEUS, CAROLUS. Hortus Cliffortianus. 1737.
191. ———. Species Plantarum. 1753.
192. LOBELIUS, M. DE. Stirpium Adversaria Nova. 1570.
193. LUDWIG, C. G. De vegetatione plantarum marinarum. 1736.
194. LUETZENBURG, PHILIPP VON. Estudo botanico do nordeste do Brazil. Rio de Janeiro, Minist. da Viação e Obras, Publ. 1922–23.
195. LUNDEGÅRDH, H. Ekologiska och fysiologiska studier pa Hallands Väderö. Bot. Not. 1: 39–52. 1919.
196. ———. Klima und Boden in ihrer Wirkung auf das Pflanzenleben. 1925.
197. LYLE, L. Distribution of the marine flora of the Channel Islands compared with that of the coasts of western Europe. Jour. Ecol. 11: 77–92. 1923.
198. MACCAUGHEY, VAUGHAN. The strandflora of the Hawaiian Archipelago. Bull. Torrey Bot. Club. 45: 259–277. 1918.
199. MACDOUGAL, D. T. Botanical features of North American deserts. Carnegie Inst. Washington Publ. 1908.
200. MACDOUGAL, D. T. and collaborators. A cycle of the Salton Sea. Veröffentl. Geobot. Inst. Rübel. Zürch. Heft 3. 1925.
201. MAHESHWARI, P. Contributions to the morphology of Boerhaavia diffusa. Journ. Indian Bot. Sci. 9: 42–61. 1930.
202. MARLOTH, R. Zur Bedeutung der Salz abscheidenen Drüsen der Tamariscineen. Ber. Deut. Bot. Ges. 5: 319–324. 1887.
203. MARSILLI, L. F. COMTE DE. Histoire physique de la mer. 1715.
204. MARTIUS, K. F. P. VON. Flora Brasiliensis. 1840–1906.
205. MASON, EDNA. Notes on the presence of mycorrhiza in the roots of salt marsh plants. New Phytol. 27: 193–195. 1928.
206. MAXIMOV, N. A. Physiologisch-okologische Untersuchungen über die Dürreresistenz der Xerophyten. Jahrb. Wiss. Bot. 62: 128–144. 1923.
207. ———. The physiological basis of drought-resistance of plants. 1926.
208. MCCREA, R. H. The salt-marsh vegetation of Little Island, Co. Cork. Jour. Ecol. 14: 342–346. 1926.
209. MIKI, SHIGERU. On the sea-grasses in Japan. Bot. Mag. Tokyo 47: 842–862. 1933; 48: 131–142. 1934.
210. MILLER, EDWIN C. Plant Physiology. 1938.
211. MÖBIUS, M. Die Erforschung der Pflanzen des Meeres. Ber. Senckenberg Naturf. Ges. 55: 107–179. 1925.
212. MÖNKEMEYER, WILH. Die Laubmoose Europas. [In Rabenhorst, Kryptogamenflora. 1927.]
213. MONTFORT, C. Die Wasserbilanz in Nahrlösung, Salzlösung und Hochmoorwasser. Zeits. Bot. 14: 97–109. 1922.
214. ———. Physiologische und pflanzengeographische Seesalzwirkungen. I Einfluss ausgeglicher Salzlösungen auf Mesophyll-und Schliesszellen; Kritik der Iljinschen Hypothese der Salzbeständigkeit. Jahrb. Wiss. Bot. 65: 502–550. 1926; 66: 902–946. 1927; 67: 105–173. 1927.
215. ———. Ueber Halobiose und ihre Aufstufungen. Flora 121: 434–502. 1927.
216. ———. Assimilation und Stoffgewinn der Meeresalgen bei Aussüssung und Rückversalzung. Ber. Deut. Bot. Ges. 49: Gen-vers. Heft. (49)–(66). 1931.

217. ————. Die Trockenresistenz der Gezeitenpflanzen und die Frage der Uebereinstimmung von Standort und Vegetation. Ber. Deut. Bot. Ges. **55**: (85)–(95). 1937.

218. ————, und J. Brandrup. Physiologische und pflanzengeographische Seesalzwirkungen. II. Ökologische Studien ueber die Keimung und erste Entwicklung bei Halophyten. Jahrb. Wiss Bot. **66**: 902–945. 1927; **68**: 801–842. 1928.

219. Martensen, H. Der Formenschatz der nordchilenischen Wüste. Abh. Ges. Wiss. Göttingen. Math.-physik. Kl. 1927.

220. Mullan, D. P. On the occurrence of glandular hairs (salt glands) on the leaves of some Indian halophytes. Jour. Indian Bot. Soc. **10**: 184–189. 1931.

221. ————. Observations on the water-storing devices in the leaves of some Indian halophytes. Jour. Indian Bot. Soc. **10**: 126–133. 1931.

222. ————. Observations on the biology and physiological anatomy of some Indian halophytes. Jour. Indian Bot. Soc. **11**: 103–118, 285–302. 1932; **12**: 165–182, 235–256. 1933.

223. Muncher, W. C. The effect of transpiration on the absorption of salt by plants. Am. Jour. Bot. **9**: 311–329. 1922.

224. Neuwohner, W. Der tägliche Verlauf von Assimilation und Atmung bei einigen Halophyten. Planta **28**: 644–679. 1938.

225. Nichol, E. D. T. The ecology of a salt marsh. Jour. Mar. Biol. Ass. U. K. 1935.

226. Nichols, G. E. The vegetation of Connecticut. VI. The plant associations of eroding areas along the seacoast. Bull. Torrey Bot. Club **47**: 89–117. 1929.

227. ————. The vegetation of Connecticut. VII. The plant associations of depositing areas along the coast. Bull. Torrey Bot. Club **47**: 511–548. 1920.

228. Nienburg, W. Zur Okologie der Flora des Wattenmeeres. Wiss. Meeresunters, Kiel **20**: 146–152. 1927.

229. ————, und E. Kolumbe. Zur Ekologie der Flora des Wattenmeeres. Das Neufelder Watt im Elbmundungsgebiet. Wiss. Meeresunters, Kiel **21**: 77–82. 1931.

230. Nitzschke, H. Die Halophyten im Marschgebiet der Jade. Vegetationsbilder 14 Heft 4. 1921/22.

231. Nordhagen, R. Vegetationsstudien auf den Insel Utsire im westlichen Norwegen. Bergens Museums Aarbog Nat. 1920/21.

232. Norgaard, O. Studer over naturforholdene i vestlandske fjord. I Hydrografi. Bergens Museums Aarbog. 1903.

233. Oliver, F. W. The bouche d'Erquy in 1906. New Phytol. **5**: 189–195. 1906.

234. ————. The shingle beach as a plant habitat. New Phytol. **11**: 23–99. 1912.

235. ————. Marine littoral plant and animal communities of New Zealand. Trans. New Zeal. Inst. **54**: 496–506. 1923.

236. Oltmanns, F. Ueber die Bedeutung der Konzentrationsänderungen des Meereswassers für das Leben der Algen. Sitzungsber. Kgl. Akad. Wiss. Berlin. 1891.

237. ————. Kultur und Lebensbedingungen der Meersalgen. Jahrb. Wiss. Bot. **23**: 349–441. 1892.

238. ————. Notizen ueber die Algenflora bei Warnemünde. Arch. Freunde Nat. Mecklenburg **47**: 97–106. 1893.

239. ————. Notizen ueber Kultur und Lebensbedingungen der Algen. Flora **80**: 38–55. 1895.

240. ————. Morphologie und Biologie der Algen. 1923.

241. Ørsted, A. S. Iagttagelser over vegetatione fordelingi Øresundet. Forh. Skand. Naturforsk. **3**: 621–622. 1842.

242. OSBORN, T. G. B. On some halophytic and non-halophytic plant communities in arid South Australia. Trans. & Proc. Roy. Soc. So. Austr. 47: 388–399. 1923.
243. ————, AND WOOD, J. G. On the zonation of the vegetation in the Port Wakenfield district, with special reference to the salinity of the soil. Trans. & Proc. Roy. Soc. So. Austr. 47: 244–261. 1923.
244. OSTENFELD, C. H. Preliminary remarks on the distribution and biology of the Zostera of the Danish Seas. Bot. Tidskr. 27: 123–125. 1905.
245. OSTERHOUT, W. J. V. On the importance of physiologically balanced solutions in plants. I. Marine plants. Bot. Gaz. 42: 127–134. 1906.
246. ————. Tolerance of fresh water by marine plants and its relation to adaptation. Bot. Gaz. 63: 146–149. 1917.
247. PACKARD, A. S. JR. The sea-weeds of Salt Lake. Am. Nat. 13: 701–712. 1897.
248. PAINE, J. A. Regents' Report. New York State. 1865.
249. PEKLO, J. Bemerkungen zur Ernährungsphysiologie einiger Halophyten des Adriatischen Meeres. Oesterr. Bot. Zeits. 62: 47–52. 1912.
250. POMA, G. L'influence de la salinité de l'eau sur la germination et la croissance des plantes halophyles. Bull. Acad. Roy. Belg. Sci. V 8: 81–92. 1922.
251. PORTER, H. C. Die Abhängigkeit der Breitling-und Unterwasserflora vom Wechsel des Salzgehaltes. Diss. Rostock. 1894.
252. PRAT, HENRI. Florule halophytique de la Grève de Trois-Pistoles. Nat. Canad. 60: 9–16. 1933.
253. PRAT, S. Some observations on *Caulerpa prolifera*. Stud. Plant. Physiol. Lab. Prague 2: 36–42. 1924.
254. PRIESTLEY, J. H. The halophilous formation of the left bank of the Severn Estuary. Bristol Nat. Soc. 3: 9–16. 1911.
255. PRODAN, GIJULA. Oecologia plantelov halofile din romãnia, comparate cu cele din Ungaria si sesul Tisei din regatul SHS. Bull. Infrom. Grav. Bot. Univ. Cluj. 2: 1–17, 37–52, 101–113, 1922.
256. RAUNKIAER, C. The life of plants and statistical plant geography. 1934.
257. REEDE TOT DRAKENSTEIN, H. A. VAN. Hortus Malabaricus. 5 Pl. 13: 6 Pl. 31–37. 1685–1686.
258. REICHE, K. Grundzüge der Pflanzenverbreitung in Chile. 1907.
259. REINKE, J. Die ostfriesischen Inseln. Studien über Küstenbildung und Küstenzerstörung. Wiss. Meeresunters. Kiel. 10: 1–79. 1909.
260. RICHTER, A. Die physiologischen Grundlagen der Wiederstandfähigkeit der Pflanzen im südosten Russlands. I Zur Frage der Wiederstandfähigkeit gegen Salze. Arb. Abt. Ang. Bot. Landw. Versuchsstat. Saratow. nr. 38. 1926.
261. REIHER, O. Zur Physiologie der Diatomeen. Sitzungber. Akad. Wien. Math. Nat. Kl. 1906.
262. RICHARDS, H., AND SPEOHR, H. Basis of succulence in plants. Bot. Gaz. 67: 405–409. 1919.
263. RICOME, M. H. Influence du chlorure de sodium sur la transpiration et l'absorption de l'eau chez les végétaux. Compt. Rend. Hebdom. Sci. Paris 137: 141–149. 1903.
264. ROSENBERG, O. Ueber die Transpiration der Halophyten. Svenska Vet. Akad. Förhandl. 54 (9). 1897.
265. RÜBEL, E. Pflanzengesellschaften der Erde. 1930.
266. ROUSSEAU, J. Les tourbières littorales: indice de la submersion de la côte sud-est de la Nouvelle-Écosse. Nat. Canad. 64: 197–206. 1937.
267. RUHLAND, W. Untersuchungen ueber die Hautdrüsen der Plumbaginaceen. Ein Beitrag zur Biologie der Halophyten. Jahrb. Wiss. Bot. 55: 409–471. 1915.

268. RUMPHIUS, G. E. Herbarium Amboinense 3: 102–126, Pl. 68–84. 1743.
269. SALISBURY, E. J. The soils of Blakeney Point. A study of soil reaction and succession to the plant covering. Ann. Bot. 36: 391–406. 1922.
270. SAMPAIO, ALBERTO JOSÉ DE. Phytogeographia do Brazil. 1934.
271. SCHAFFNER, J. H. Note on the salt marsh plans of northern Kansas. Bot. Gaz. 25: 255–260. 1898.
272. SCHENK, H. Ueber die Luftwurzeln von *Avicennia tomentosa* und *Laguncularia racemosa*. Flora 72: 83–88. 1889.
273. SCHIMPER, A. F. W. Die indo-malaische Strandflora. 1891.
274. ———, AND FABER, F. C. VON. Pflanzengeographie auf physiologischer Grundlage. 3 Aufl. 1935.
275. SCHRADER, H. A. Ueber Palla's Halophyta mit besonderer Rücksicht auf die Gattungen *Salsola* und *Suaeda*. Schrad. Neue Jour. Bot. 3 (3-4) 58–92. 1809.
276. ———. De Halophytes Pallasii, respectu imprimis ad Salsolam et suaedam habito commentatir. Comm. Rec. K. Ges. Wiss. Göttingen. 1 (phys.) 16 p. III. 1811.
277. SCHRATZ, E. Beitrage zur Biologie der Halophyten. 80: 112–142. 1934; 81: 59–93. 1934; 83: 133–189. 1936; 84: 593–638. 1937.
278. ———, UND BEILER, A. Beobachtungen über die Salzverhältnisse und Halophytenvegetation in Adolf-Hitler-Koog. Ber. Deut. Bot. Ges. 55: 506–513. 1937.
279. SCHTSCHERBAK, J. Ueber die Salzauscheidung durch die Blätter von *Statice Gmelini*. Ber. Deut. Bot. Ges. 28: 30–34. 1910.
280. SCHULZ, A. Die Verbreitung der halophylen Phanerogamen in Mitteleuropa nordlich der Alpen. 1901.
281. ———. Die halophilen Phanerogamen Mitteldeutschlands. Zeits. Naturw. 75: 252–293. 1903.
282. ———. Ueber das Vorkommen von Halophyten in Mitteldeutschland auf kochsalzfreien Boden. Ber. Deut. Bot. Ges. 36: 410–413. 1918.
283. SCHÜTTE, H. Die Entstehung der Seemarschen. Arch. Deut. Landw. Ges. Heft. 178. 1911.
284. SCHWEINFURTH, G. The flora of the desert surrounding Helwân. Pay may, Helwân and the Egyptian Desert. 1901.
285. SEGERSTAD, H. A. Sydsvenska florans växtgeografiska huvudgrupper. Diss. Upsala, Malmö. 1924.
286. SEN-GUPTA, JATIS. Ueber die osmotische Werte und den Chloranteil in Pflanzen einiger Salzgebiete Bengals. Ber. Deut. Bot. Ges. 56: 474–485. 1938.
287. ———. Der histochemische Nachweis von Natrium bei einigen Pflanzen von Bengal. Ber. Deut. Bot. Ges. 56: 486–494. 1938.
288. SERNANDER, R. Studier över lavarnas biologi. I. Nitrofila lavar. Svensk Bot. Tidskr. 6: 803–883. 1912.
289. ———. De nordeuropoeiska havens vaxtregioner. Svensk Bot. Tidskr. 11: 72–124. 1917.
290. SETCHELL, W. A. Geographical distribution of the marine spermatophytes. Bull. Torrey Bot. Club 47: 563–579. 1920.
291. ———. Marine plants and Pacific palaeogeography. Proc. Pac. Sci. Congr. 5th. Canada, 1933. 4: 3117–3131. 1934.
292. SHANTZ, H. L. Indicator significance of the natural vegetation of the southwestern desert region. Jour. Agric. Res. 28: 721–802. 1924.
293. SHARP, H. S. The physical history of the Connecticut shoreline. State Conn. Geol. & Nat. Hist. Survey Bull. 46. 1929.
294. SKOTTSBERG, C. The geographical distribution of vegetation in South Georgia. Geogr. Jour. (London) 20: 498–502. 1902.
295. ———. Vegetationsbilder aus Feuerland, von den Falkland-Inseln und Sudgeorgien. Vegetationsbilder Heft 3/4. 1907.

296. ———. Studien über das Pflanzenleben der Falklandinseln. [In O. Nordenkjöld, Wissenschaftliche Ergebnisse der schwedische Sud-polar-Expedition. 1901–1903. Bd. 4 Botanik. Stockholm. 1919.]
297. ———. Botanische Ergebnisse der schwedische Expedition nach Patagonien und dem Feuerlande. 1910–1923.
298. SLOANE, HANS. Catalogus of plants of Jamaica. 1696.
299. ———. A voyage to the islands of Madeira, Barbados, Nieves, St. Christopher and Jamaica. 1707.
300. SMITH, C. A., AND HUBBARD, C. E. Notes on African grasses. IX. Some saline-loving grasses occurring in South Africa. Bull. Misc. Inf. Kew. 83–87. 1929.
301. STAHL, E. Einige Versuche ueber Transpiration und Assimilation. Bot. Zeits. **38**: 268–292. 1880.
302. STEINER, MAXIMILIAN. Zur Ökologie der Salzmarschen der nordöstlichen Vereinigten Staaten. Jahr. Wiss. Bot. **81**: 94–202. 1935.
303. STERNER, R. The continental elements in the flora of South Sweden. Geogr. Annal. 221–228. 1922.
304. STEWART, ALBAN. Some observations concerning the botanical conditions of the Galapagos Islands. Wisc. Acad. Sci. Trans. **18**: 272–340. 1916.
305. STOCKER, O. Beiträge zum Halophytenproblem. Ökologische Untersuchungen der Strandund Dünenpflanzen des Dars. (Vorpommern). Zeits. Bot. **16**: 289–330. 1924; **17**: 1–24. 1925.
306. ———. Ökologische-pflanzengeographische Untersuchungen an Heide-, Moorund Salzpflanzen. Naturwiss. **12**: 634–646. 1924.
307. ———. Die ägyptisch-arabische Wüste. Vegetationsbilder **17** (5/6). 1926.
308. ———. Das Wadi Natrun. Vegetationsbilder **14** (1). 1927.
309. ———. Der Wasserhaushalt ägyptischer Wüsten- und Salzpflanzen vom Standpunkte einer experimentellen und vergleichenden Pflanzengeographie aus. Bot. Abh. Heft. 13. 1928.
310. ———. Das Halophytenproblem. Ergeb. Biologie **3**: 265–353. 1928.
311. ———. Transpiration und Wasserhaushalt in verschiedenen Klimazonen. II. Untersuchungen in der ungarischen Alkalisteppe. Jahr. Wiss. Bot. **78**: 751–856. 1933.
312. STOCKER, O. Salzpflanzen. [In: Handwörterbuch der Naturwissenschaften. 2 Aufl. Bd. 4. 1933.]
313. STOKLASA, J. Ueber den Einfluss des Jodiums auf das Wachstum und die Zellvermehrung der Halophyten. Biochem. Zeits. **211**: 213–228. 1929.
313. SVENSON, H. K. Studies on interior distribution of maritime plants. Rhodora **29**: 41–48, 57–72, 87–93, 105–114. 1927.
314. SWARTZ, O. Observationes botanicae quibus plantae indiae occidentalis. 1791.
315. TACKE, B. Ueber die Beziehungen zwischen dem Gehalt des Bodens and Kochsalz und dem Pflanzenwuchs. Abh. Nat. Ver. Bremen **26**: 503–509. 1927.
316. TAKENOUCHI, M. Investigations on the relation between plants and their surrounding conditions by the quantitative method. II. On the ecological value of the result of studying the interrelation between water absorption and the transpiration of plants. Bull. Sci. Fak. Terkult. Kyushu Imp. Univ. **2**: 213–230. 1927.
317. TAYLOR, WM. RANDOLPH. The marine algae of Florida with special reference to the Dry Tortugas. Carnegie Inst. Washington Publ. 1928.
318. TERRAS, J. H. Notes on the salinity of the cell-sap of halophytes with

relation to that of the soil solution in which they grow. Proc. Scot. Micr. Soc. 1934.

319. THORODDSEN, TH. An account of the physical geography of Iceland with special reference to the plant life. [In: The Botany of Iceland 1: 1914.]
320. TOBLER, FRIEDRICH. Biologie der Flechten. 153–155. 1925.
321. TOLMATCHEW, A. Über die Verbreitung einiger Strandhalophyten in der Arktik Bot. Not. 213–227. 1934.
322. TORREY, JOHN. Flora of New York. 1843.
323. TOTTINGHAM, W. E. A preliminary study of the influence of chlorides upon the growth of certain agricultural plants. Jour. Am. Soc. Agron. **11**: 1–32. 1919.
324. TROLL, W. Ueber die sogenannten Atemwurzeln der Mangroven. Ber. Deut. Bot. Ges. **48**: Gen. Vers. Heft. (81)–(99). 1930.
325. ————, UND OTTO DRAGENDORFF. Ueber die Luftwurzeln von *Sonneratia* L.f. und ihre biologische Bedeutung. Planta **13**: 311–473. 1931.
326. TROTTER, A. Rilievi ecologici intorno alla flora ed alla vegetazione della salina di S. Giorgio presso Taranto. Ann. R. Ist. Sup. Agr. Portici **3**: 120–135. 1932.
327. UPHOF, J. C. TH. The plant formations on the coral reefs along the northern coast of Cuba. Am. Jour. Bot. **11**: 409–416. 1924.
328. ————. Biologische Beobachtungen an *Batis maritima*. Oesterr. Bot. Zeits. **79**: 356–367. 1930.
329. ————. Vegetationsbilder der Östliche Staaten von Nordamerika. Vegetationsbilder **21** Heft 1/2. 1930.
330. ————. Batidaceae. Pflanzenareale **3** Heft 2. 1931.
331. ————. Vegetation der Niederlande. Vegetationsbilder **22** Heft 7. 1932.
332. ————. Die holzigen Halophytenvereinen des Küstengebietes Floridas. Mitt. Deut. Dendrol. Ges. **47**: 39–53. 1935.
333. ————. Die nordliche Verbreitung der Mangroven im atlantischen Gebiet. Arch. Hydrobiol. **31**: 141–144. 1937.
334. VALENTIJN, FRANCOIS. Verhandeling der zee-horenkens en zee-gewassen in en omtrent Amboina en de nabijgelegen eilanden. 1754.
335. VALLIN, H. Ökologische Studien über Wald-und Strandvegetation. Lunds Univ. Arskrift. 1925.
336. VESQUE, J. De l'influence des matières salines sur l'absorption de l'eau par les racines. Ann. Sci. Nat. Bot. VI. **9**: 5–11. 1882.
337. VILBOUCHEVITCH, J. Renseignements sur des plantes de terrains salants, saltbushes, kendyr, dy Turkestan. Bull. Sp. Nat. Acclim. France **40**: 365–374. 1893.
338. VOLKENS, G. Die Flora der Ägyptisch-Arabische Wüste. 1887.
339. WALTER, H. Die Anpassungen der Pflanzen an Wassermangel. Das Xerophytenproblem in Kausalphysiologischer Betrachtung. Naturwiss. & Landw. Heft 9 Freising-München. 1926.
340. ————. Einführung in die allgemeine Pflanzengeographie Deutschlands. 1927.
341. ————. Ueber den Wasserhaushalt der Mangrovenpflanzen. Ber. Schweiz. Bot. Ges. **46**: 217–228. 1936.
342. ————, UND M. STEINER. Die Ökologie der ost-afrikanische Mangroven. Zeit. Bot. **30**: 65–193. 1936.
343. WALTON, J. A Spitzbergen salt-marsh; with observations on the ecological phenomena on the emergence of land from the sea. Jour. Ecol. **10**: 109, 121. 1937.
345. WARBURG, O. Einige Bemerkungen über die Litoral-Pantropisten. Ann. Jard. Not. Buiten. Suppl. II. 1898.

346. ———. *Rhizophora mangle* L. Tropische Fragmente. Bot. 'ahrb. 4: 517–548. 1893.
347. WARMING, E. Halofyt-studier. D. Kgl. Denske vid. Selsk. 6 raekke. Naturvid. og Math. Afd. VIII. 4. 1897.
348. ———. Dansk Plantevolkst. I. Strandvegetation. 1906.
349. ———, UND GRAEBNER, P. Lehrbuch der ökologischen Pflanzengeographie. 4 Aufl. 1930–32.
350. WEAVER, JOHN E., AND CLEMENTS, FREDERIC E. Plant Ecology. 2 Ed. 1938.
351. WEEVERS, T. De invloed van het zoutgehalte op de verspreiding van verschillende halophyten. Nederl. Kruidk. Arch. 46: 898–912. 1936.
352. WERDERMANN, E. Die Pflanzenwelt Nord- und Mittelchiles. Vegetationsbilder 21: Heft 6/7. 1931.
353. WETTSTEIN, RICHARD VON. Vegetationsbilder aus Sudbrasilien. 1904.
354. WHERRY, EDGAR T. Plant distribution around salt-marshes in relation to soil acidity. Ecology 1: 42–48. 1920.
355. WIEHE, P. O. A quantitative study of the influence of tide upon populations of *Salicornia europaea.* Jour. Ecol. 23: 323–333. 1935.
356. WINKLER, HANS. Einige Bemerkungen über Mangroven-Pflanzen im Hamburger Botanischen Garten. Ber. Deut. Bot. Ges. 49: 87–102. 1931.
357. WOHLENBERG, E. Ueber die tägliche Leistung von *Salicornia herbacea* L. im Haushalte der Watten. Wiss. Meeresunters. Abt. Helgoland. 19 Abh. 3. 1933.
358. ———. Das Adelpolster und die Enstehung einer charakterischen Abrasions-form im Wattenmeer. Wiss. Meeresunters. Abt. Helgoland 19 Abh. 4. 1933.
359. WORONICH, N. N. Zur Biologie der bittersalzhaltigen Seen in der Umgebung von Pjatigorsk. Arch. Hydrobiol. 27: 628–653. 1926.
360. WULFF, H. D. Karyologische Untersuchungen an der Halophytenflora Schleswig-Holsteins. Jahrb. Wiss. Bot. 84: 812–840. 1937.
361. YAPP, A. H., JOHNS, O., AND JONES, N. T. The salt-marshes of the Dovey estuary. Jour. Ecol. 5: 65–82. 1917.
362. ZANARDINI, G. A. N. Notiz e intorno alle cellulari marine delle lagune de litorale di Vinezia. Atti R. Ist. Ven. 1846–47: 185–265. 1847.
363. ZELLNER, J. Zur Chemie der Halophyten. Monats. Chem. 47: 611–618. 1927; 56: 197–199. 1930; 59: 305–306. 1932.

-E BOTANICAL REVIEW

V
II FEBRUARY, 1941 No. 2

-IE NATURE OF DISEASE RESISTANCE IN PLANTS. I.[1]

S. A. WINGARD
Virginia Agricultural Experiment Station

INTRODUCTION

t
o st in the nature of disease resistance in plants was origi- .i· the pioneer studies of De Bary (51) and Ward (275, isease nature of parasitism of plants by fungi. The practical mmu- f disease resistance in plants was immediately recog- d im- on (183), Duggar (62), Freeman (73), Biffen (19, es on lsson-Ehle (181), Vavilov (261, 262) and others. astance, in 1908 stated: "When we introduce into our ture varieties possessing a degree of natural immunity and avoid both the loss from disease and the necessity for the r less expensive treatment by sprays and other means, a economic gain will be secured."

ese pioneer workers realized also that the occurrence in nature ase resistance or immunity in plants offered a means of im- g crop plants through breeding, for Orton in 1908 also made lowing statement: "The productiveness of all our crops must eased and their quality improved. The workers in this field ake note not only of problems of heredity but of pathology as They must know the nature of the disease, its governing s, and the type of resistance involved in order to adopt the promising lines of approach in breeding." Duggar (62) in also emphasized this fact when he said: "There is insufficient edge respecting the nature of response in the cells of the host vaded by the parasite."

sis extensive literature on the general subject of disease re- plants has been developed over the last thirty-five years; ch of this literature consists of reports of

only casual observations which can not be used as a basis ing definite conclusions. On the other hand, many worker this period have directed their efforts specifically to the s disease resistance, and as a result our knowledge in this fi been greatly advanced. But it requires only a casual review literature to reveal the fact that disease resistance in plants very incompletely understood. In the present paper the hopes to give a comprehensive review of the literature on the of natural disease resistance in plants but no claim will be for an exhaustive review. The literature on this subje already been reviewed (31, 35, 37, 113, 270, 293).

For convenience in this discussion, the terms "disease resis and "immunity" will be used to denote different degrees same thing; that is, it is possible to have various degrees of d resistance, with immunity being used to designate complete d resistance. The discussion will deal primarily with natural i nity or disease resistance in plants, but the subject of acquire munity can not be entirely disregarded, especially since stud immunity (if such exists in plants) should throw light on n immunity. The literature on this subject has also been rev (37, 38, 116).

Orton (183), in his pioneer work, realized that in order t derstand the nature of disease resistance one must first be fa with parasitism. He said: "Before we can fully underst nature of disease resistance it will be necessary to study nomena of parasitism, a subject which involves some of t complicated of the many interrelations between organism nature has to offer us." We are thus fortunate to have literature on host-parasite relations reviewed and discussed t cytological standpoint by Allen (2, 3, 4, 5), Rice (211, 2 Wellensiek (282), and from a physiological standpoint by (31). These reviews contain a vast amount of information interest to all who are interested in the nature of parasit disease resistance in plants. One can not read these revie out fully realizing the intricate and complex nature of host- relations.

TYPES OF DISEASE RESIS

From only a casual review of the litera

has been recognized for a long time, as shown by Duggar's statement in 1911: "Every disease produced by an organism presents the finite problem of certain complex relations between the cells of the host and those of the parasite." Freeman (73) in the same year emphasized the fact that the essential character of true resistance, as pointed out by Ward (275), Salmon (222), Biffen (21), Orton (186) and others, lies in a protoplasmic activity and is thus independent of inoculation accidents. He further states: "A variety may escape a disease through certain peculiarities of physiologic host activities, of meteorological conditions, or even of certain morphological host characters."

The following statement by Orton (185) in 1908 shows that he clearly recognized the existence of several types of disease resistance: "The typical form of disease resistance involves a specific reaction on the part of the host cell against a true parasite, a character developed in nature in the evolution of the species and strengthened in cultivated plants through the work of the breeder. Less important from the breeder's standpoint are plants resistant through (*a*) structural differences, (*b*) disease endurance, and (*c*) disease avoidance. The evidence indicates that the resistance is due to a specific protective reaction of the host cell against the parasite. So in plants the evidence leads us to believe that more is involved than the acidity of the cell sap or the chemotactic effect of sugars or other food substances. The first group is the most important, relating as it does to diseases due to the most highly developed parasites, such as the rusts, mildews and other injurious fungi. The evidence indicates that the resistance is due to a specific protective reaction of the host cell against the parasite." Orton was sure that the reaction between host cell and fungous parasite in plants was the same as that described for some forms of immunity in man and the higher animals in which substances in the blood serum neutralize the toxin excreted by the invading bacteria and result in the destruction of the latter. He stated, however, that the evidence in plants leads us to believe that more is involved than the acidity of the cell sap or the chemotactic effect of sugars or other substances. He said: "The delicacy of the reaction may be better understood if we recall the fact that it is adjusted to repel specific parasites. A plant resistant to one disease may be quite susceptible to another. General hardiness is also another matter. A plant

may be resistant to cold and yet extremely susceptible to the at of some parasite."

"Structural differences," Orton says, "do not seem to play muc part in enabling plants to resist the true parasite. Satisfactory demonstrations of cases where resistance to highly adapted parasites is due to thickened epidermis, development of hairs, *etc.*, are lacking. It has on the other hand been shown by Ward and Salmon that germinating spores of fungi often penetrate the epidermis of plants they can not parasitize, and are killed forthwith by the cells they attack. It is hard to understand why a thick cell wall should protect from infection a leaf which has many thousand openings as breathing pores through which a fungus might enter."

That Orton also recognized the importance of morphological resistance under certain conditions is indicated by his statement that "Resistance due to structural causes does occur in troubles due to wound parasites, a fruit or a tuber with a thick rind being thereby less liable to bruising; as there may be an indirect connection, a plant of more open habit of growth being thereby less subject to attack by fungi which require moisture for their development."

The observations made by Eriksson and Henning (67) in 189 by Ward and his students (274, 275, 276) in 1902 and 1905, an by Vavilov (262, 263, 264) in 1914 and 1919 showed that mechanical immunity, although it may have some significance in certa instances, can not be accepted as a universal phenomenon. The concluded that natural immunity does not depend on the anatomica peculiarities of plants, but on properties of their cytoplasmic cell contents, and on active resistance of host plant cells, usually accompanied by a complicated physiological reaction in response to penetration by the parasite. Other workers just as strongly contend that resistance or immunity in plants is due to morphological factors. Still others have confused "disease-enduring" and "disease-escaping" varieties with resistant or immune varieties.

In the literature on disease resistance in plants one finds, in addition to examples of disease-enduring and disease-escap varieties, numerous instances in which plants have been show be resistant to a particular disease because of either their chem or morphological properties, or both. It is not always easy to s-tinguish between morphological, or mechanical, immunity nd physiological, or chemical, immunity, as will be brou out the

later. Since this paper is intended as a general review erature on the nature of disease resistance and immunity ₃, it is felt that the subject should be dealt with in a general so as to include all types of information that might serve to a clearer understanding of the nature of disease resistance.

MISCONCEPTIONS OF DISEASE RESISTANCE

Freeman (73) in 1911 pointed out the importance of correcting popular misconceptions regarding the nature of disease resistance, o der that the work of the plant pathologist might not be hampered by unearned criticism and loose terminology. He stated that: "In the popular mind and even in a good deal of scientific literature any variety that escapes a particular disease is classed as a esistant variety." Orton (185) had already pointed out the differences between what he termed disease-escaping, disease-enduring, disease-resisting, and immune varieties. Orton said: "Disease endurance sometimes results from the ability of the plant to grow in spite of an attack, either through exceptional vigor or through a hardier structure, as in the case of certain melons which bett r survive the attacks of leaf-blight because the leaves do not dry out as quickly as do those of the ordinary melons. Drought-resistant plants are often disease-enduring. Watermelons from semi-arid Russia were for this reason the last to succumb to the wilt disea when planted in our southern States." "Finally," said Ortc , "we have disease-escaping varieties. Such, for example, are the extra early cowpeas which mature before the season for wilt and oot-knot to develop. These varieties which escape disease th gh earliness or lateness are often really very susceptible. The ır Ohio and other early potatoes, which commonly mature fore the appearance of the late blight disease, are among the first to succumb to this disease if planted so late as to be still immature wher the moist weather of the late summer of early fall enables late bligh to spread."

Fr nan goes ahead to say: "There is little difference in the real esi e powers of oat varieties to the common oat rusts, and w rower is told that an oat variety is resistant because it usually es the rust through earliness of ripening, he is led into a seriou istake which will sooner or later throw discredit on the work o e pathologist. The essential character of true resistance

as pointed out by Ward, Salmon, Biffen, Orton, and others lies in protoplasmic activity and is independent of inoculation accidents. A variety may escape a disease through certain peculiarities of physiologic host characters. The latter is well illustrated by the following experiments of the writer. A variety of barley was grown in different soils provided by Harter of the U. S. Department of Agriculture during his alkali soil investigations. These soils varied from the normal garden soil to those containing about 2% of alkaline salts. Plants in the different soils when inoculated by spraying methods in the greenhouse showed different amounts of rust, in general those in the stronger alkaline soils showing less rust. The latter, however, when attacked exhibited large and vigorous growths of the rust. The smaller percentage of pustules was undoubtedly due to the greater development of "bloom" on the barley foliage when grown in the strong alkali, resulting in the rolling off of the water drops, and hence in the loss of the inoculating material. Harter has shown that the plants in the different soils exhibited measurable differences in the thickness of the outer epidermal walls. The bluish color of the plants in the alkaline soils and the greater tendency of the water drops to run off were quite pronounced. There seems to be no reason for assuming that there was any difference in real rust resistance. Again Ward has shown that wheat rust uredospores may send germ tubes into the stomata of plants of many different genera other than barberry, wheat and other grasses, and that they may even produce a rudimentary mycelium, but they fail to penetrate the protoplasmic defense of the mesophyl and soon succumb.

"The economic importance of disease-enduring varieties ought not to be overlooked by working pathologists. It seems probable that exceedingly effective work in selection and breeding may be accomplished in the production of disease-enduring varieties. The best varieties of the hard spring wheats commonly used in the north central states, both of blue stems and fifes, come under this class in regard to stem rust, since not one of them possesses any appreciable amount of resistance. They can endure light or even moderate attacks of rust, but all go down quite uniformly under conditions in a heavy epidemic. It is, of course, obvious that a sharp line of demarcation may not exist between disease-enduring and disease-resistant varieties. For practical purposes of selection and breed-

ing, however, well marked resistance can readily be detected under proper experimental conditions.

"Immune varieties possess perfect resistance. Their production will probably always remain rare in comparison with those possessing only partial resistance or high powers of endurance. In this discussion the term 'resistance' is used only in reference to true resistance."

Butler (35), Brick (23) and others have also emphasized the importance of distinguishing between disease resistance and disease escape. According to Butler, it is necessary to distinguish between avoidance of disease, endurance of disease, and true immunity or resistance to disease. He gives several instances of the ways in which plants may avoid a disease to which they are not in any way truly resistant. They may be grown in areas with conditions of climate which the parasite can not stand, it being shown that many of our most important cultivated plants have a wider range than their parasites; or the date of sowing may be altered to a period when the temperature or humidity is unsuitable for germination of the spores of the parasite; or varieties may be grown which mature quickly before the parasite can do them much damage. Cases are given of successful endurance of the attacks of a plant parasite. These are concerned mostly with the vigor of the plant, and can be modified by different methods of cultivation and manuring. True resistance to disease differs from the above in depending on some structural or physiological characters of the plant which prevent successful invasion by the parasite. He cites specific cases in which structural characters are important, but he regards the physiological characters which influence immunity as the most important class of factors in the majority of cases.

In 1919 Brick pointed out that susceptibility of plants to disease is not due to degeneration, old age, and other similar causes enumerated by practical horticulturists, but usually is the result of certain differences in morphological or anatomical structures of the host itself. He believed that increased acid, sugar and tannin content also have a bearing upon the resistance of a variety to parasites. He says some plants escape disease because their season of blossoming or maturing does not coincide with development of the parasite organism. These statements are supported by numerous citations from the literature dealing with susceptibility and resistance to rusts, smuts and other diseases.

It is obvious from the above that any one interested in the development of disease-resistant plants should first attempt to get a clear understanding of what constitutes true disease-resistance, especially of what is involved in the particular case in which he is interested.

RELATION OF ENVIRONMENT TO DISEASE DEVELOPMENT

There is evidence that environmental factors play an important rôle in the development of plant diseases. In fact, so obvious is the relation of weather conditions to the relative damage to plants from disease that the grower often believes that unfavorable rainfall or temperature is the direct and only reason for his trouble. It was pointed out by Smith (234) in 1911 that the most important and the most complex problems for long-time research include the critical study of the relation of environment to parasitism. Such factors as the temperature, moisture, fertility and reaction of the soil have been shown to have a marked effect on disease development. Light and the temperature and humidity of the air also play an important rôle in many cases. The effect of environmental conditions on the development of disease may be either direct or indirect, or both, and may vary greatly with different diseases. There is an extensive literature dealing with the relation of weather to plant diseases, as indicated by the bibliography prepared by Wilson (285) which includes nearly 4,000 references. Since this literature has been so recently reviewed by Foister (72), only a few cases will be given here.

Effect of soil temperature. Jones and his associates (133*a*) in Wisconsin were the first to perfect technique for studying the influence of soil temperature on soil-borne diseases, and for a review of their work the reader is referred to their papers (128, 133*a*). However, some of their results as well as those of others will be given here to illustrate the point.

Jones (128), for example, in 1917 tells how he was confronted with this problem in Wisconsin in 1915 and 1916. He states: "During 1915 and 1916 we had under critical study two fungous diseases of the commonest garden crops, *viz.;* the late blight of the potato (*Phytophthora infestans*) and the yellows disease of cabbage (*Fusarium conglutinans*). Of these two summer seasons, 1915 was cool and moist and 1916 exceptionally hot and dry. In

1915 the late blight fungus, stimulated by favoring weather, destroyed some millions of dollars worth of potatoes with the worst outbreak for at least a decade. As a result, almost every lot of seed potatoes in the state carried the infection to the fields in 1916, yet the dry heat held the parasite so completely in check that the expert mycologist had to search the potato fields of the state with a magnifying glass to find a single incipient development of the disease. By way of contrast in 1915, alongside these sick potato fields in Wisconsin, the cabbage crop was everywhere vigorous, even on the worst Fusarium 'sick' soils, whereas under the dry heat of 1916 these same cabbage fields were swept by the yellows disease as if by fire." Any one dealing with plant diseases can parallel such experiences.

Jones' (128) interest was first aroused in the possible relation of soil temperature to infection of grain smut in 1893 upon finding that oat smut during several years was, as a rule, less abundant in Vermont than in the western States. Other workers, among them Hecke (107) in 1909 and Bartholomew and Jones (17) in 1923, were able to show that soil temperature is of much importance in connection with infection of oats by loose smut, *Ustilago avenae*. It was found that high soil moisture combined with low soil temperature at planting time reduced the amount of smut infection. Heavily inoculated oats were sown in the field in the spring in wet, cold soil. The soil temperature ranged during the germination period from 2° to 24° C., averaging about 11°; the moisture averaged about 45% to 50% of saturation and reached 70% at times. The resulting oat plants showed less than 0.1% of smut at maturity, whereas seed from the same lot grown under experimentally controlled conditions favorable to infection produced 100% smutted plants.

Edith S. Jones (125) studied the influence of temperature upon germination of oat smut spores in beef broth, and upon growth of the smut fungus on potato dextrose agar. The results were similar for the two processes, the extreme range being about 5° to 32° C., wtih fair development from 15° to 28° C., and the best about 20° C.

Jones (126) further states that in 1905 he was again impressed with the probable importance of soil temperature as a factor in plant pathology when he learned how much less prevalent potato scab (*Actinomyces scabies*) was in northern Europe than in

America, a fact that seemed more probably related to soil temperature than to any other variable factor. In northern Europe potato scab is generally regarded as a minor disease, in spite of the highly intensive culture of the crop and the abundant use of stable manure from animals fed on cull potatoes, which is contrary to what happens in America with continued potato culture on the same soil. Jones, McKinney and Fellows (130) later through controlled experiments showed that potato scab develops in greatest abundance at soil temperatures ranging from about 20.5° to 23° C. with 22° as the general optimum. These data are in line with what actually happens under field conditions.

Fellows (70) in 1926 showed that the potato tuber is most susceptible to scab infection during a limited period following the "setting" of the tubers and that practically all the tubers on a plant are "set" at about the same time. Infection takes place through the unsuberized lenticels and stomata, and there are relatively more unsuberized lenticels and stomata on rapidly growing tubers than there are on tubers which grow more slowly at the extremely high soil temperatures. The rapidly growing tubers were found to be more susceptible to scabbing than those which grow more slowly. This explains why the optimum soil temperature for scabbiness is lower than the optimum temperature for the growth of the scab parasite.

As a result of their studies on the Fusarium diseases of truck and cereal crops, several writers (188, 114, 288, 142, 101) suggested the importance of soil temperature as a factor influencing the distribution or severity of these soil-infesting parasites. Later studies on these and other soil-borne diseases have shown that soil temperature is a dominant factor in the development of these diseases.

Prominent among the diseases in which soil-temperature has been shown to play a dominant role are cabbage yellows (127, 93, 253, 250), flax wilt (251, 252), tomato wilt (47, 65), tobacco root-rot (119), stem canker of potato caused by *Rhizoctonia solani* (213, 214), seedling blight of cereals caused by the Fusarium stage of *Gibberella saubinetii* (54), *Helminthosporium* diseases of wheat and barley (155), take-all disease of wheat (50, 156, 157), *Diplodia* seedling blight of corn (117), pea blight and root-rot (131, 133), onion smut (269), white rot of onion (271), and tobacco black shank (254, 257, 135). The results of these workers show

that variation in a single factor of environment, such as soil temperature, may profoundly influence the occurrence or severity of disease. For example, with the worst degree of soil infestation and with other environmental conditions favorable, it was shown that moderate variations in soil temperature may at the one extreme lead to complete destruction of the crop by certain diseases, or at the other extreme may result in practical freedom from disease development. This has been exemplified by such varied disease types as onion smut, potato stem canker, tobacco root-rot, cabbage yellows, *etc.* Geographic distribution of certain diseases can be explained on the basis of soil temperature relations.

Soil temperature can also be correlated with seasonal occurrence of diseases, especially of the vascular Fusarium diseases (*e.g.*, cabbage yellows, flax wilt, tomato wilt). Invasion proceeds from the soil through the roots and advances through the vascular elements to the aerial parts. At all stages progress is dependent upon favorable temperature relations. The general curve of disease development rises gradually to its optimum, which is about 28° C., then makes a sharp drop as the temperature rises above this. In all cases there is an upper as well as a lower point beyond which the host may survive, in even the worst infested soil, free from evidence of the disease. In other words, the host has a wider growth-temperature range than has the potential parasite.

As pointed out by Jones and his associates (133*a*), one should not lose sight of the importance of other environmental factors, but in the case of the vascular Fusaria no other variable factor was found to exert so important an influence as temperature. This dominant influence of soil temperature as compared with other factors seems to hold for some other diseases, such as those caused by *Thielavia basicola* and *Rhizoctonia solani.* On the other hand, temperature may be only one of the important influential variables with a disease like common scab of the potato where the influence of soil moisture or of soil reaction may also be highly important. With still other diseases, as club root of cabbage, it has been shown that temperature may have only a minor influence, while other factors such as soil reaction or soil moisture may be dominant.

Effect of air temperature, humidity and light. Evidence presented above shows that soil temperature and moisture are influential factors in the development of diseases that attack subter-

ranean parts of the plant. Evidence will now be presented to show that temperature, light and humidity may also influence the development of diseases that attack aerial parts. Clayton (47), in studying Fusarium wilt of tomato, found that both soil and air temperature are important factors. The optimum soil temperature for growth of the tomato plant and for development of the wilt disease is about 28° C. With the soil temperature above 33° C. or below 21° C., the disease was practically eliminated, although the tomato plant itself grew fairly well at a somewhat wider range in either direction. Clayton also studied the effect of air temperature on tomato wilt, using three different temperatures in combination with different soil temperatures. If the soil was kept cool (17° C.) or hot (33° C.), the fungus could not establish itself in the root tissues. If, on the other hand, the soil was favorably warm (27° C.), the Fusarium invaded the root system and pushed upwards into the basal part of the stem. If, then, the air temperature also was favorable (27° C.) the parasitic invasion continued upward throughout the aerial parts of the plant leading promptly to wilting and death. When the soil was held at a favorable temperature (27°) and the air at an unfavorable temperature (17°), the root system was invaded but the progress of the fungus in the aerial portion of the stem was so inhibited that no wilting resulted. Further study showed the stem tissues in such cases to be free of the fungus. The fungus stopped within one-half inch above the soil line.

Rivera (216), from his studies on mildew epidemics on wheat, oak and rose, concluded that plants are most susceptible when partially wilted, and that maximum turgidity brings about resistance. Fruit pathologists consider bitter rot of apple and brown rot of peach as hot weather diseases, and apple scab and cedar rust as cool, wet weather diseases. Humidity at a particular season of the year is essential for development of these diseases. More recently, Wolf *et al.* (289), Armstrong and Sumner (11) and Dixon *et al.* (58) have presented results to show that temperature and humidity are dominant influential factors in the development of downy mildew of tobacco caused by *Peronospora tabacina.* Dixon *et al.* summarize their results on downy mildew of tobacco as follows: "Sporulation is favored by long periods of saturation at times when the sky is overcast. Abundant sporulation occurs within a

range of temperature from 42° F. to 63° F. and is most abundant at 56° F. If the temperature is outside this range, either less than 42° F. or greater than 63° F., sporulation is sparse. Few, if any, sporangia are formed above 68° F. or below 36° F. Maximum temperature above 90° F., accompanied by intense sunshine, inhibits abundant sporulation even when night conditions are favorable".

Shaw (229) found that increased resistance in apple shoots to fire blight was favored by loss of soil nutrients, either low or high soil temperature, high atmospheric temperature and low soil moisture. Increased susceptibility was favored by intermediate or opposite conditions. Brief exposure of plants to a high atmospheric humidity very greatly reduced fire blight resistance.

Temperature and humidity are controlling factors in the development of bean anthracnose (16, 64). The same holds true for many other diseases of truck and field crops.

Much work has been done on the effect of environmental conditions on the development of the rusts of cereals, beans and other plants. That temperature exerts an important influence on rusts has been observed by a number of investigators (18, 39, 74, 75, 94, 99, 99*a*, 100, 108, 115, 124, 139, 148, 162, 167, 191, 192, 196, 227, 239, 246, 275, 276, 278, 279, 280). The effect of temperature on the development of rust seems to be indirect so far as the rust fungus is concerned, that is, whatever makes for the betterment or detriment of the host is likely to be reflected to some extent in the activity of the rust. The rapidity of rust development, as measured by length of the incubation period, increases with rise in temperature until a point is reached at which metabolism of the host is impaired. Waterhouse (278) has shown that seasonal variations in climate in Australia exert a marked influence on the rust reactions of wheat, oats and barley seedlings grown in the greenhouse. This was true especially for stem rust on seedlings of wheat varieties which showed the heterogeneous type of reaction to stem rust. For example, the Joanette strain of wheat was resistant to physiologic form 1 of stem rust in the winter, susceptible to it in summer, and gave a heterogeneous reaction in spring and fall, but the reaction of this host to physiologic forms 2 and 7 of stem rust throughout the year was not significantly changed. Gordon (94) and Johnson (124) have confirmed the results of Waterhouse. In

Gordon's studies the Joanette strain of wheat showed a striking difference in its reactions to physiologic forms 1, 3, 4 and 5 at a low (57.4° F.) and at a high (75.4° F.) temperature. However, the effect of temperature (57.4°, 64.8°, 71.9° and 75.4° F.) on the reactions of Joanette strain and the other hosts studied to physiologic forms 2, 6, 7 and 8 was negligible.

Fromme (75) found the incubation period for *Puccinia coronata* to be twelve days with the temperature at 14.5° to 21° C., and seven days at 20° to 30° C., and Mains (150) found that of *P. Sorghi* to be thirteen days at 13° C., and seven days at 18° to 20° C., whereas at 30° C. no uredinia were developed at the end of fourteen days and only a sparse amount of mycelium. Peltier (192) found that the optimum temperature for initial infection with *P. graminis tritici* form III is about 25° C., whereas that for form IV is about 20° C. Optimum temperature for development of the disease with both forms on plants in seedling, stooling and jointing stages was between 20° and 25° C. No infection occurred at temperatures of 10° C. and below, and only a few plants of some of the differential hosts were infected at 15° and 30° C. With plants at heading stage a lower optimum temperature for infection occurred in that no rust developed at 30° C., whereas it did at 10° C. The temperatures at which wheat plants make their growth are generally the same as those at which the rust makes its best development. Inoculated plants held at 5° C. for nine weeks showed no apparent infection, but developed infection when transferred to higher temperatures.

Peturson (196), in studying the reaction of oat varieties to different physiologic forms of *P. coronata avenae,* found three varieties completely susceptible to form VII at 77° F., very resistant to the same form at 70° F., and practically immune from this form at 57° F. Melander's (162) work with *P. graminis tritici* indicated that the type of infection produced on certain varieties of wheat with some of the physiologic forms of rust could be changed by varying the temperature. At 0 to 1° C. the infection was the type produced on resistant hosts, but when the seedlings were transferred to a temperature of 20° C. the type of infection changed to that normally produced on susceptible hosts. In Germany, Gassner and Straib (86), found that wheat varieties resistant to *P. glumarum tritici* at 20° C. were severely rusted at slightly lower tem-

peratures. In Canada, Newton, Johnson and Brown (179) tested a number of wheat varieties and found them all resistant to stripe rust at 78° F., but that many varieties were completely susceptible at 54° F. Gassner and Straib (87) found that certain varieties were highly resistant to leaf rust (*P. triticina*) form XIV at 20° C. but completely susceptible to the same form when cultured at lower temperatures.

The exact manner in which environmental factors influence the disease has been determined in a few cases. Noteworthy among these is that of seedling blight of wheat and corn (*Gibberella saubinetti*) which was investigated by Dickson *et al.* (55, 56). The same strain of *G. saubinetii* attacks varieties of both wheat and corn, but with the difference that wheat is attacked at high temperatures (16° to 18° C. according to the variety) but not at low temperatures; whereas corn is attacked at low temperatures (8° to 16° C.) but not at high temperatures. This indicates a striking correlation between resistance and a type of metabolism in the plant which is in the main determined by the prevailing temperature. At low soil temperatures, the starch of the wheat endosperm is hydrolyzed much more rapidly than is the protein, with the result that the seedling is rich in sugar but poor in nitrogen. The cell walls, therefore, thicken rapidly by deposit of cellulose material upon the original pectic framework, and on that account become much less susceptible to fungal attack. On the other hand, at high temperatures both the starch and the protein are rapidly hydrolyzed, the seedling is richer in soluble nitrogen, growth is much more rapid and the cell walls remain much longer in the primary pectic condition. They are thus more susceptible to fungal attack. Corn seedlings naturally prefer a higher temperature for growth than wheat seedlings and thus their behavior in relation to temperature is the converse of that described for wheat. At high temperatures, favorable to corn, the cell walls of the seedlings are of a resistant and somewhat suberized type, whereas the unmodified pectic type of cell wall is produced at low temperatures. Dickson *et al.* (57) later found that corn seedlings of resistant varieties grown at 12° to 16° C. contained approximately 20% more uronic acids (associated with polyglucuronides) than those of susceptible varieties grown at the same temperatures. Similarly, Gaumann (90), showed that attack by *Fusarium herbarum* on wheat was

correlated with the presence of xylan and other hemicelluloses; the higher the percentage of xylan, the more readily did the enzyme of the fungus destroy the cell walls. Gassner and Franke (89) found that the more severe infection of wheat by *P. glumarum* and *P. triticina* at low temperatures was correlated with increase of albumen at low temperatures.

Reddy (207) found in the case of *Basisporium gallarum* in corn that low temperatures did not allow active translocation to start, as was described above for seedling blight. The fungus thus enters the seed at low temperatures because, translocation from the embryo not having started, there is still a neutral reaction which is not toxic to the fungus. At higher temperatures, translocation commences at once with germination, the tissues become acid and therefore toxic to the fungus, conferring an apparent resistance to the plant. Some of the newer corn varieties are adapted to growing at lower temperatures by having early translocation from the embryo. Such varieties are resistant to the disease even at the lower temperatures.

Conant (49) has shown why tobacco is more resistant to *Thielavia* root rot at higher soil temperatures. The amount of infection and the severity of the *Thielavia* root rot disease of tobacco is most marked at soil temperatures of 17° to 23° C. At a temperature below 15° C. the disease is much reduced, but such temperatures are too low for growth of the plant. On the other hand, at a temperature of about 26° C. destructiveness of the parasite rapidly lessens until at about 29° to 30° C. it produces very little injury, and at 32° C. practically no infection occurs. In culture the *Thielavia* fungus grows best at 28° to 30° C., and relatively poorly at 17° to 23° C. at which root rot infection is most severe. Conant found resistance in tobacco to root rot caused by *Thielavia basicola* to be definitely correlated with the ability of the host to develop a cork layer beneath the point of infection. This reaction on the part of the host is accelerated by raising the temperature of the soil in which the plant is rooted, until at 26° to 30° C. and above, all varieties of tobacco become resistant to attack by the root rot fungus. The protective reaction occurred at all temperatures with the naturally resistant varieties, whereas with the susceptible varieties the corking off of the fungus occurred only at the higher temperatures. The same phenomenon was found by Fahmy (68) to occur in the sore-shin disease of cotton. The re-

covery of cotton plants from attack by *Rhizoctonia solani,* which is brought about by raising the temperature, is accomplished by the walling off by a corky layer of the cavity formed by the fungus. A review of the literature, however, does not indicate that this is the only type of response an infected plant may make under varying soil temperatures.

Thomas and Ark (249) point out that development of the bacteria which cause fire blight of pears is correlated with high humidity. The nectar of the pear flowers is a favorable medium for multiplication of the bacteria under conditions of high humidity and may thus serve as a source of infection for visiting bees which spread the disease to other flowers; whereas at low humidity the bacteria do not multiply rapidly. This is because at high humidity the sugar content of the nectar is only 1 or 2%, whereas at low humidity it may run as high as 55%. The high sugar content not only prevents development of the bacteria but also destroys their virulence. Shaw (230) found that the degree of susceptibility of apple and pear twigs to infection by *Erwinia amylovora* is correlated with the intercellular humidity of the twigs. The twigs showed high susceptibility and high intercellular humidity when the environmental moisture content was high, and low susceptibility and low intercellular humidity when the environmental moisture content was low. With the average intercellular humidity between 97 and 98.5%, the plants were immune or only slightly susceptible; with it above 99.5%, they were highly susceptible; and with the intermediate intercellular humidities, the plants were intermediate in blight susceptibility.

Clayton (45, 46) has found that water soaking of leaves plays an important rôle in the development of wildfire and blackfire of tobacco. Johnson has confirmed Clayton's results and shown further that this situation is not peculiar to tobacco or to the organisms that cause tobacco wildfire and blackfire. Plants normally immune to infection by an organism may become susceptible to attack when the tissues are watersoaked. (290, 122).

Effect of soil reaction. The effect of soil reaction on plant pathogens is apparently not so great as that of temperature and moisture. Nevertheless, soil reaction plays an important rôle in the development of many diseases. For example, *Fusarium* wilt of tomatoes (233), black scurf of potatoes (166), club root of cab-

bage (40), and *Pythium* damping-off of legume seedlings (166) are favored by high soil acidity. Potato scab, caused by *Actinomyces scabies* (224), *Thielavia* root-rot of tobacco (9, 10, 10*a*), *Phymatotrichum* root-rot of cotton (244) and powdery mildew of cowpeas (30) are hindered by high soil acidity.

Sanford found that a soil pH of 5.2 would prevent development of potato scab but that severe scab might be expected in soils ranging from a strongly alkaline reaction to at least pH 5.4. Lime and barnyard manure increased scab as much as 40% in some cases. Brown found that cowpeas grown in soil with a pH of 4.8 did not have mildew; those on soil with a pH of 6.4 had some mildew, and those on soil with a pH of 7.5 had a severe dose of mildew on both leaves and stems. Taubenhaus *et al.* (244) have reported a new *Fusarium* wilt of cotton in Texas that is important only on soils more acid than pH 6.5 to 7.0. In Egypt there is a *Fusarium* wilt of cotton that is important only on alkaline soils. *Phymatotrichum* root rot of cotton (35) is destructive only on soils more alkaline than pH 6.5 to 7.0. When it is found in acid soils, pH 5.5 to 6.5, it causes negligible damage as compared to losses ranging from 20 to 100% in neutral or alkaline soils. The extremes for infection by this disease are pH 4.1 and 8.9. Optimum for infection is pH 7.0. Anderson (10) and others have shown that tobacco root rot infection depends directly upon the reaction of the soil. Root rot is checked by a pH of 5.5 but becomes injurious as the acidity of the soil decreases below this point.

According to Butler (35), larch canker is worst on calcareous soils and rare on siliceous types. So also the *Oidium* and mildew of the vine are said to be more prevalent on soil rich in lime than in sandy loam because the acidity of the sap is reduced by the former (Averna 1910). On the other hand, *Iris* varieties that prefer a limestone soil are liable to be severely attacked by *Heterosporium gracile* when grown in soil deficient in lime (206).

Effect of Nutrition. Discussion here will be limited to the effect of nutrition on the parasitic diseases of plants with the so-called nutritional or physiological diseases purposely omitted.

It is rather generally believed that the health and vigor of an organism, whether plant or animal, and its susceptibility to disease are antithetic variables; that is, as one increases or is increased the other diminishes or is diminished correspondingly. This view is

expressed by Zinnser (294), Kolmer (136) and Jones (126). In direct contrast with this view, workers in the rusts of higher plants have on several occasions been prompted to generalize directly to the opposite effect—that host vigor and susceptibility to disease are not antithetic, and not independent, but parallel variables. Arthur (14), after many years' study on plant rusts, states that the association of the parasite and host is so intimate that as a rule the vigor of the parasite is directly proportional to the vigor of the host. Sheldon (231), summarizing his studies on asparagus rust, concluded that lowered vitality of the host does not favor infection but that whatever affects the growth of the asparagus has a like effect on the rust. Stakman (238), from his extensive studies on cereal rusts, has on several occasions expressed similar views. For instance, he concluded that whatever is conducive to vigorous development of the host is ordinarily conducive to vigorous development of the parasite also.

There are others who believe that excess nitrogen tends to predispose plants to disease, whereas potassium and phosphorus, especially potassium, tend to make them more resistant. The literature on the subject, however, shows that one should not generalize as to the effect of nutrition on plant diseases, because different host plants and pathogens are affected differently by environmental conditions, and what is true of one disease or of diseases caused by one group of fungi, such as the rusts, will not necessarily hold true for diseases caused by other pathogens.

The greater susceptibility to rust of wheat grown on highly fertile soil has been noted repeatedly. Little (145) as early as 1883 stated that high manuring, especially with nitrogenous fertilizers, predisposes wheat plants to rust. Bolley (32) confirmed Little's statement in 1889. Freeman and Johnson (74), in their review of the cereal rust problem in the United States in 1911, say: "It is now well established that where there is an excess of nitrogen in the soil, other things being equal, grains are more severely attacked by rust than crops on soil containing less nitrogen. Where barnyard manures have been applied heavily the result is similar, and where grains are grown after clover, beans, or vetch, rusts may be expected. In fact it may be generally stated that where soils are rich in nitrogen, producing rank and succulent plant growth, rust attacks will be most severe. In general, a rust attack is most viru-

lent on a healthy plant." Similar views have been expressed by others (6, 12, 240, 281, 102, 88, 205, 209, 152, 118, 172, 21, 48, 265, 111, 243).

Many workers feel that the effect of nitrogenous fertilizers on rust susceptibility in cereals is largely indirect. Stakman in 1913 made experiments on the effect of nutrient salts on the development of *P. graminis* on wheat plants in soil and sand cultures and found that excessive amounts of such fertilizers as nitrogen and phosphorus salts had no direct effect on the immunity or susceptibility of wheats. Vavilov (261) concluded that the increased susceptibility of wheat to *Puccinia triticina,* when grown in soil fertilized with nitrogen, was due to increased development of leaf surface rather than to any change in real resistance. Armstrong (12) in 1922 pointed out that wheat fertilized with nitrate of soda was delayed in maturity and opportunity, for development of *P. glumarum* was increased. He thus attributed the effect of nitrogen to a lengthening of the growing period rather than to an actual increase in susceptibility. Gassner (85) came to the same conclusion with respect to the rusts of wheat, oats, barley, rye and corn. Freeman and Johnson (74) in 1911 were in accord with this view.

Increased susceptibility to disease due to nitrogenous nutrition is not confined to the rusts. Marchal (151) found that infection of lettuce by mildew (*Bremia lactucae*) was favored by nitrogen and phosphate and retarded by an excess of potassium. Jones (126) mentions that high fertilization, especially with nitrogenous fertilizers, lowers the power of the potato plant to resist blight and rot. McCue (154) observed that tomato plants treated with phosphatic fertilizers developed less leaf blight than control plants, while plants on nitrogen and potash plots which at the same time gave the greatest yields, were more heavily infected than the controls. Thomas (247) obtained evidence of increased susceptibility to leaf spot (*Septoria apii*) of celery plants as a result of excess applications of nitrate of soda. Zobel (295) reported that reduction of manure in the trenches and top dressing the soil with Kainit greatly reduced *Septoria* leaf spot of celery in England. Levine (141) noted that crown gall in beets (bacterial disease) developed more rapidly and to larger size on roots grown in a highly manured soil. Stewart (241), Stewart, V. B. (242), Reimer (208), and others have observed that apple and pear trees on plots highly fertilized with

nitrogen and those making the most vigorous growth are most affected with fire blight. Link and Wilcox (143) in controlled pot experiments were able to show that application of nitrates with particular light, temperature and moisture conditions favored a metabolic status resulting in succulence and susceptibility to infection of Stayman apple shoots by *Erwinia amylovora*. Non-applications of nitrates under the same conditions favored early cessation of meristematic activity, resulting in woodiness and resistance to infection.

Other diseases in which increased nitrogenous fertilizer has been shown to increase susceptibility to infection include citrus canker (191*a*), angular leaf spot of tobacco (77), mildew of wheat (256), eye-spot disease of sugar cane (140), coffee leaf disease (35), gray rot in grapes caused by *Botrytis cinerea* (53), potato blight (153), gooseberry mildew (223), corn smut (226, 13, 197, 138), flax rust (228), wet rot of potatoes (69), apple scab (123), *Pythium* root rot of wheat and sugar cane (260) and sweet corn wilt (236).

Examples from agricultural practice of a similar effect of nitrogeneous manuring could be multiplied, and the phenomenon has been studied under the more rigorous conditions of water culture. Spinks, for example, concluded from the results of his studies on mildew and yellow rust of wheat and mildew of barley that these diseases are increased by providing the plants with large amounts of available nitrogen. Certain mineral fertilizers, especially phosphates and potash salts, on the contrary, decreased the susceptibility to these diseases, but they did not counteract the effect of large quantities of nitrogenous fertilizers. Lower susceptibility did not appear to be due to a lack of food material available for the fungus in the host, because the plants rendered less susceptible by applications of larger quantities of phosphates and potash salts seemed to be as healthy and well grown as those receiving no such additions.

Trelease and Trelease (256) grew Marquis wheat in different culture solutions and inoculated it with mildew, *Erysiphe graminis*. Indices of susceptibility were yields and development of mildew per unit area of host. Susceptibility of the wheat was markedly influenced by the composition of the culture solution, the least susceptible plants being secured with a solution having the following molecular proportions of main salts: 90% KH_2PO_4, 5% $Ca(NO_3)_2$, and 5% $MgSO_4$. The most susceptible plants were

those grown in a solution containing 5% KH_2PO_4, 47.5% $Ca(NO_3)_2$, and 47.5% $MgSO_4$. They concluded that some factor or factors, other than plant growth, or host vigor determined susceptibility of wheat to *Erysiphe graminis*. Turner (258) found that barley and corn, high in nitrogen, were readily attacked by mildew. Spencer and McNew (236), in studying wilt of sweet corn seedlings caused by *Phytomonas stewarti*, found that seedlings dwarfed by high concentrations of nitrogen, phosphorus or potassium were more severely infected than those grown at concentrations more conducive to rapid growth. Seedlings deficient in either nitrogen or phosphorus were only slightly infected, whereas potassium-deficient seedlings were severely infected. Seedlings supplied with a nitrogen-deficient solution developed small necrotic lesions but little or no wilting of invaded leaves. With high nitrogen, wilting was so intense that about one-half of the seedlings died within two weeks after inoculation. In the potassium-deficient seedlings, infection was more severe than in seedlings receiving small amounts of potassium. Later work by McNew and Spencer (159) showed that the amount of nitrogen in the tracheal sap of maize directly affected the growth of *Phytomonas stewarti*, the organism causing wilt of sweet corn. They attribute this direct effect to the fact that the wilt organism lives almost exclusively in the tracheal tubes during early stages of its invasion and, therefore, depends upon the materials in the transpiration stream for its sustenance. McNew has shown elsewhere (158) that virulent strains of the wilt bacterium always use inorganic nitrogen and that the ability to use such nitrogen is closely associated with virulence of the parasite. This explains why the severity of invasion by the bacterium is directly correlated with the total amount of nitrogen supplied the seedlings rather than with the growth of the host resulting from such applications. The bacterium grew in trachael sap that contained 20 to 40 p.p.m. of nitrogen, but did better in sap with about 200 p.p.m.

McNew and Spencer point out that their observations on the effect of nitrogen on the host-parasite complex present only one aspect of the problem of plant nutrition in relation to disease. They state that such a direct effect on the parasite may not apply to other diseases in which the parasite is established in tissues other than the tracheal tubes. However, they call attention to the fact

that Nightingale (180) has offered evidence that the invasiveness of *Erwinia amylovora* in the cortical tissues of apple twigs is correlated with the amount of organic nitrogen present, which in turn can be controlled by the amount of inorganic nitrogen supplied the tree.

From the foregoing statements it would seem safe to conclude that nitrogenous fertilizers predispose plants to disease. This, however, may not be true in every case, for there are reports to the contrary. Rolfs (218), for example, in studying the bacterial shot-hole disease of peaches, plums and cherries, states that trees in good condition are as a rule not seriously injured by the disease. He concludes that thorough cultivation of the orchard and use of nitrogenous fertilizers are most valuable in controlling this disease. Valleau (259) comes to the same conclusion with respect to controlling the shot-hole disease. Rolfs (217), in studying the angular leaf spot disease of cotton, found that the plants in complete fertilizer plots showed much less injury than those in the check plots. The plots which received two top dressings of nitrate of soda, one in May and one in June, were best. The plants in the high potassium plots showed even less injury than those in the high nitrogen plots. Liberal applications of barnyard manure and a complete fertilizer followed by top dressings with nitrate of soda gave the best results.

Phosphorus and potash are reputed to have an influence in increasing disease resistance, but this action is best established for potash, both by practical experience and scientific experiments. Some of this evidence has been presented in connection with the discussion on nitrogen and need not be repeated. Butler (35) states that it is a common practice of growers of plants under glass, such as tomatoes in England, to check the ravages of various parasites by potash manuring. Phosphatic manuring (284) has been used with success in the control of *Thielavia* root rot of tobacco and ginseng. Hassebrauk (102) found that potassium and phosphatic salts tend to induce resistance to cereal rusts. As a result of strong potassium deficiency, a type of wheat possessing moderate resistance shifted to one of high susceptibility. However, the immune and highly susceptible varieties were little influenced by these nutrients. Gassner and Hassebrauk (85) later confirmed Hassebrauk's results. According to Sharvelle (228), flax rust on a normally

susceptible variety was not significantly changed by nutrients, but the development of rust on normally resistant varieties was greatly increased by applications of excess nitrogen or phosphate; it was somewhat suppressed by applications of potash. Russell (219) states that potash fertilizers in northern Ireland decrease the liability of flax to attacks of the wilt organism. Neal (177), Young and his associates (290, 291, 292), Miles (169) and others have shown that potash fertilizers have a very definite beneficial effect in controlling cotton wilt caused by *Fusarium vasinfectum*. Cralley (49*a*) has found that the stem rot of rice (*Leptosphaeria salvinii*) can be largely controlled by potassium. Severe infection was obtained when the plants were grown in unbalanced solutions, high in nitrogen and low in potassium. With solutions in which the nitrogen and phosphorus were held constant, stem rot severity decreased as the potassium level in the solution was increased. Other workers (221, 210, 176) have reported a similar result with this disease. Moss *et al.* (173) state that "Under some conditions the use of potash seems to control partially several of the leaf-spot diseases of tobacco, including wildfire and blackfire, especially when used at a liberal rate. On those plots which were fertilized with a mixture carrying heavy rates of ammonia with little or no potash the various leaf-spot troubles have been more prevalent, causing serious damage; but with more potash added to the fertilizer there has been much less damage from leaf spot."

Application of potash has also been reported as having a beneficial effect on the control of rose mildew in Australia (106), on *Rhizoctonia* root rot of jute in India (71), on corn root rot in the U. S. (112) and on stripe rust of wheat (174).

Phosphorus has been reported as having a beneficial effect on the prevention of *Septoria* leaf spot of tomato (182), eye spot disease of sugar cane (140), dodder on clover and vetch (164), downy mildew of cabbage (41), and corn root rot (112). Hoffer and Trost found that corn root rot (*Gibberella saubinetii*) was much worse where either potash or phosphorus was deficient. It was later found that this disease can be largely controlled by application of potash, phosphorus and lime. Reimer (209) in 1903 concluded that acid phosphate would inhibit development of cereal rusts on plants grown in soils in which there was not too much nitrogen. Montemartini (172) stated that starved plants were immune from rust and that phosphates always increased resistance.

Instances have been reported (35) where phosphorus rendered the plant more susceptible to the disease. In the disease caused by *Sclerotinia libertiana,* a fungus which attacks a number of garden plants, superphosphate has been found to reduce resistance under certain conditions. This fungus requires an acid medium for its parasitic growth and appears to have the power of secreting acid. In lime soils the acid tends to be neutralized and plants grown in such soils resist the disease. Heavy manuring with superphosphate restores the acidity of the plant tissues and thus enables the fungus to thrive. In lettuce mildew (*Bremia lactuca*) phosphates have also been found to reduce the resistance of plants grown in water culture. Millikan (170) reports that development of powdery mildew in wheat was greatly reduced by a deficiency of phosphorus in the plant, whereas deficiencies of other minerals had no effect on this disease.

Other elements such as calcium and boron have been shown to have an influence on disease resistance. Gigante (92) found in pot tests in the greenhouse with *Puccinia triticina* and in field tests with *P. glumarum* that additions of sodium borate to the soil not only increased the resistance of wheat to these fungi but also had a stimulating effect on the growth of the plants. Weiss (281) in 1924 reported that increase in calcium was not only associated with lowered susceptibility to wheat rust infection but with a materially lower water requirement in rusted as well as in rust-free plants. Thomas and Muller (248) found that hydrated lime reduced infection of celery by *Septoria* leaf spot. Millikan (170), in sand culture experiments, however, found that an unbalanced nutrition exercised a profound effect on the reaction of the susceptible variety of wheat, Free Gallipoli, to flag smut (*Urocystis tritici*). A deficiency of calcium in the plant resulted in very marked reduction in development of the disease. When deficiency of calcium was sufficiently great, the disease was completely inhibited. Deficiencies of nitrogen and potassium also resulted in decreases in the disease, which, however, were not so marked as those associated with calcium deficiency. Phosphorus deficiency resulted in significant increase in flag smut disease, while magnesium deficiency had no effect. Addition of twice the normal amount of calcium to the nutrient solution significantly increased the severity of flag smut but addition of four times the normal amount had no signifi-

cant effect. It was possible to relate the above changes in the reaction of the plants to flag smut with changes in their calcium content induced by the mineral treatments.

Excess calcium tended to reduce flag smut resistance in the resistant Ghurka variety but none of the treatments applied increased the smut susceptibility to a point where it was in any way comparable with that of the susceptible control plants grown under the same conditions.

In cereal rusts it is the general consensus of opinion that nitrogenous fertilizers increase the susceptibility of the plants to infection, while potassium and phosphorus salts tend to decrease it. Some investigators attribute this effect principally to changes in the chemical composition of the plants but there is apparently not much evidence to support this view. Other workers (225, 220, 171, 194) think the effect is rather on the mechanical composition of the cell walls. Schindler says that acid phosphate seems to cause development of stronger tissues, and Russell concludes that the walls of plants fertilized with excess nitrogen are thinner than they normally would be, and parasitic fungi therefore can penetrate more easily. Miyake and Adachi agree with Russell regarding the effect of nitrogen. They report that potassium fertilizers strengthen the cell walls and thus increase resistance to disease. It should be of interest in this connection to review the work of Hursh (115*a*) who found that stem-rust resistance in certain varieties of wheat was due to the high amount of sclerenchymatous tissue in the stem. This was explained by the fact that the rust fungus can live only in the chlorenchyma, and as the only tissue in the stem which contains chlorophyll is in the collenchyma, it is obvious that the amount of collenchyma in the stem would determine to a very large extent the development which the rust fungus can make. Hursh found that the relative proportion of sclerenchyma to collenchyma in a given wheat variety could be altered by the use of fertilizers. Excessive fertilization with nitrogen had a tendency to decrease the amount of sclerenchyma in proportion to collenchyma, and thus render plants heavily fertilized with nitrogen more susceptible to stem rust than those which had not been so fertilized. Conversely, phosphates and potassium fertilizers may cause an increase in the amount of sclerenchymatous tissues and thus limit the area in which the rust mycelium can grow. Hursh

was very careful to call attention to the fact that although the morphological structure of the wheat stem may be changed by fertilizer applications or other environmental conditions, we should not overlook the fact, however, that the fundamental protoplasmic resistance of the plant probably is not changed. The individual cells of the different tissues probably are no more susceptible in the plants fertilized with nitrogen than in those not so fertilized, but the number of cells in which the rust fungus can develop is greater in the nitrogen fertilized plants.

Stakman and Aamodt (240), after making a thorough study of the effect of artificial and natural fertilizers on the development of stem rust on resistant and susceptible varieties of wheat, concluded that the degree of physiologic susceptibility of susceptible and resistant varieties apparently is not changed directly by the use of different fertilizers, although morphologic resistance may be changed slightly. The heavier stem rust infection on plants receiving heavier applications of nitrogenous fertilizers apparently was an indirect effect due to increased density of stand and delayed maturity, which made conditions for infection more favorable and lengthened the time during which the plants could become infected. These workers, however, obtained some evidence that the amount of orange leaf rust may be increased by use of nitrogenous fertilizers. The results of Spinks (237), Voelcker (265), Armstrong (12), Vavilov (261) and Gassner (85) are in accord with those of Stakman and Aamodt. They, too, found that highly resistant varieties remained resistant even when heavily fertilized with nitrogenous fertilizers.

Raines (205) studied the factors governing the virulence of *P. coronata*, *P. secalina*, *P. triticina* and *P. sorghi* and concluded that "A more catholic point of view in pathologic thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of diseases and in defining the practical problem of disease prevention and control". Raines' conclusion, in general, corroborates the evidence given by others (276, 14, 232, 91, 238, 75, 148) that the vigor of various rust parasites is likely to be directly proportional to the vegetative vigor of the host. This can be best explained on the basis of the obligate nature of the rust parasite. It can thus be

readily seen that, although it may be fairly safe to generalize with respect to the effect a fertilizer element may have on the reaction of plants to a certain class of diseases, it is never safe to follow such a generalization in predicting the reaction of a particular plant to a specific disease, as a result of the application of a certain kind of fertilizer. This, however, is as it should be since the relations of the host and parasite are apparently as different for the groups of saprophytes, semisaprophytes and obligate parasites as are the modes of life of these organisms. It is possible, according to Thomas (247), to arrange parasitic fungi in an intergrading series on the basis of the completeness of adaptation to the host, from a form such as *Botrytis*, which habitually kills the host cells before it reaches them and is probably never in intimate contact with the cell, to a form such as the seed fungus of *Lolium temulentum*, which has reached such a high degree of adjustment with the host that it is perpetuated entirely in the mycelial form through the seed of the host and perhaps never kills any of the host cells. Furthermore, as pointed out by Lutman (146) in view of the apparent importance of nitrogen in the construction of amino acids; of phosphorus in that of nucleic acid; of magnesium in that of protoplasm, especially in the fruiting stage, and of chlorophyll; of calcium in carbohydrate removal, in the neutralization of cell acids, or the deposition of new cell walls; of potassium in carbohydrate assimilation and enzyme activity; it would seem that some of the effects of the lack of any one of these, on the one hand, or of its superabundance, on the other would, be noted not only in the general habit of growth but also in the finer details of the structure of the cells themselves, and would thus influence the susceptibility of the cellular tissues to infection by disease organisms.

This matter seems to be well summed up by Petri (195) who concludes that the conditions of nutrition of a plant determine in a large measure the degree of its resistance or susceptibility to pathogens; its histological or morphological structure or properties and the function of the organs and tissues may be modified in such a way as to retard or hasten the attack of the pathogen. Nitrogen, in general, tends to increase crop susceptibility to adverse causes, phosphorus and potassium to increase resistance against the same causes. However, no general law can be established, since in one case the conditions may make a plant susceptible and in another

resistant. The inherent reaction of the soil and its amendments may bring about a similar result. The plant nutrients used may act directly on the mechanism of resistance to pathogens, or indirectly and as a modification of the functions of growth in any given habitat. Hence, there are so many things involved in the host-parasite relationship of disease, and the host plants and parasites concerned in the various diseases differ so greatly, that the effect of environmental conditions is apt to be different for each disease or for each class of diseases, depending on the nature of the parasitism of the causal organism. On this basis the effect of nutrition on rusts, caused by obligate parasites, would be quite different from that on leaf spots and stem cankers which are caused by facultative parasites on weak hosts.

GENETIC BEHAVIOR OF DISEASE RESISTANCE

Although the hereditary nature of disease resistance has been shown to be a common phenomenon, its genetic behavior does not appear to follow any single genetic law. However, the results of numerous investigations carried out by many workers during the last thirty-five years show that immunity and resistance are as a rule conditioned by one or more Mendelian factors. As pointed out by Walker (270), the chief limiting factors in the advancement of our knowledge of this phase of the subject have been the necessity of assuming an arbitrary division between resistance and susceptibility in analyzing the behavior of any group of plants, the present imperfect understanding of the effect of environmental factors upon the expression of resistant characters, and the existence in many cases of several distinct biologic strains of the parasite.

Studies of the inheritance of disease resistance in plants have been a fruitful field for the application of genetic principles to plant breeding, and the literature on the subject is voluminous (287, 137).

The data presented here will serve to show the diversity of results obtained thus far. Biffen (19) reported that resistance to yellow rust (*Puccinia glumarum* Eriks. & Henn.) in his cross between Rivet, a rust-resistant wheat, and Red King, an extremely susceptible one, behaved as a recessive factor, giving all susceptible plants in the F_1 generation and approximately 75% susceptible in the F_2 generation. Similar results were obtained by Biffen with

reciprocal crosses between the varieties Rivet and Red King. The character for resistance to yellow rust was recessive and followed the simple 3:1 Mendelian ratio. In two later papers, Biffen (20, 21) reports the results of further work with his wheat hybrids. These results bore out his former conclusions regarding the genetic behavior of rust susceptibility in wheat. He further asserts that "None of the cases examined up to the present time indicate that rust susceptibility itself is due to the existence of more than one factor". Biffen found that resistance to mildew (*Erysiphe graminis* DC) in barley was inherited in much the same way as resistance to yellow rust in wheat.

Nilsson-Ehle (181), in studying crosses between wheats resistant to yellow rust and varieties susceptible to this disease, found susceptibility dominant in the F_1 generation, and was of the opinion that a multiple factor explanation of the genetic behavior of resistance was the correct one in his crosses. Armstrong (12) later obtained results concerning the inheritance of resistance to this rust closely approximating those of Biffen. Vavilov (262), in crosses between Persian wheat (*Triticum vulgare* var. *fuliginosum* Al.) which was immune to mildew (*Erysiphe graminis* DC) and varieties of common bread wheats susceptible to the disease, secured F_1 hybrids which were immune to mildew.

Orton (183, 184, 185, 186), in his work from 1902 to 1911, showed that varieties of watermelon (*Citrullus vulgaris*) differed in their resistance to wilt, caused by *Fusarium niveum* (EFS). He found that an inedible variety of South African origin and the variety Stock Citron were markedly resistant to the disease. He then crossed the variety Stock Citron with the wilt-susceptible variety Eden and obtained the wilt-resistant variety Conqueror, an edible variety. This is probably the first instance known where, by hybridization, a plant variety resistant to a fusarial disease has been developed. Orton found that susceptibility was dominant over resistance in the F_1 plants of crosses of resistant with susceptible varieties. He was of the opinion that the character for resistance behaved in true Mendelian fashion. His studies (186) in crossing wilt-resistant and nematode-resistant varieties of cowpea with susceptible varieties served to confirm this view. In his cowpea crosses, Orton found disease resistance to be recessive in some cases and dominant in others. In addition to breeding a

wilt-resistant watermelon, Orton succeeded in developing wilt- and nematode-resistant varieties of cowpeas by hybridization. He had available as a parent the Iron, a variety quite immune to both wilt and nematode, but deficient in seed-producing qualities, and having a tendency to produce long vines. He wished to produce a variety with disease resistance, upright habit, leaf-holding ability, and productiveness. The Whippoorwill variety was used to supply the qualities other than disease resistance. Segregates appeared among the hybrid plants in which the disease-resistant quality was combined with the other desired properties. Orton concluded that disease resistance was a unit factor, although he did not make ratio determinations to bear out that conclusion.

Orton's pioneer work in developing wilt-resistant varieties of watermelon by hybridization has been followed up in recent years by others (198, 199, 200, 201, 165). Several wilt-resistant varieties of watermelon of good edible quality have thus been made available for commercial production by these workers in the past few years.

Tisdale (251), studying the inheritance of resistance to flax wilt (*Fusarium lini* Bolley) in crosses between resistant and susceptible varieties of flax, obtained a great variety of results grading all the way from F_1 generation plants that were wholly resistant to F_1 generations that were wholly susceptible. He explains his results on the basis of multiple factors, claiming that under normal conditions two or three factors in the homozygous condition would be enough to show apparent resistance. Under conditions exceptionally favorable for infection only those plants which have all the factors concerned in the homozygous condition would show resistance. Burnham (34), however, in studying the inheritance of resistance to flax wilt, found that susceptibility was a dominant factor, and that more than one factor was concerned with resistance. Walker (272, 273), on the other hand, has reported results from crosses between varieties of cabbage resistant and susceptible to the yellows disease (*Fusarium conglutinans*) in which yellows resistance was found to be a dominant unit character. The F_1 hybrids were disease free; and the F_2 hybrids segregated into a ratio of three resistant to one susceptible, indicating that resistance and susceptibility are allelomorphic unit characters. The F_1 plants when crossed with susceptible plants gave offspring with a ratio of

one resistant to one susceptible. Wade (266), in studying resistance to wilt (*Fusarium orthoceras* var. *pisi*) in canning peas with six different crosses, found only a single factor difference involved in resistance. Crosses of susceptible × resistant, resistant × susceptible, back-crosses of F_1 to the susceptible parent, back-crosses to the resistant parent and F_1 produced results indicative of a monohybrid difference, with resistance dominant to susceptibility.

Burkholder (33), in crosses between Well's Red Kidney, a type of bean resistant to two physiological strains of anthracnose (*Colletotrichum lindemuthianum* Bri. & Cav.), and a white marrow bean resistant to only one of these strains, obtained in the F_2, out of a total of 473 plants tested, 362 which proved resistant and 111 which proved susceptible to the disease. These numbers show almost an exact 3:1 ratio and indicate a single factor difference between the resistant and susceptible plants with respect to the one strain of anthracnose concerned in the cross. Resistance was found to be dominant to susceptibility. Later, McRostie (160) confirmed Burkholder's results. He crossed Well's Red Kidney bean, a variety resistant to both the "A" and "F" strains of the anthracnose fungus, with a selection of Michigan Robust, a variety susceptible to the "A" strain of the fungus but resistant to the "F" strain, and found that only a single factor difference existed between resistance and susceptibility in that particular cross. The ratios obtained throughout the studies between resistant and susceptible plants indicated quite clearly a single factor difference between resistance and susceptibility to the one strain of anthracnose concerned in the cross. The fact that resistance was dominant made it more difficult to isolate types which were homozygous for resistance. McRostie (161), two years later, published an article in which he gave data to show that resistance to root rot (*Fusarium Martii phaseoli* Burk) and resistance to mosaic (virus) in beans were also inherited in his crosses. He states that his results indicate that susceptibility to mosaic is partially dominant over resistance, and that susceptibility to root rot is definitely dominant over resistance. A two-factor hypothesis was advanced to account for the inheritance of susceptibility and resistance in the case of root rot and mosaic of beans. Many of his F_2 selections were shown to be accurately analyzed by their performance in the F_3 generation.

Johnson (120, 121) has studied the inheritance of resistance to black root rot of tobacco, caused by *Thielavia basicola* (B. and Br.)

Zoph. In crosses between the susceptible White Burley variety and the resistant Little Dutch variety, the first generation plants were intermediate in resistance. The second generation showed individuals of all degrees of resistance, from those even more resistant than the resistant parent to others as susceptible as the susceptible parent. Selected individuals in the third generation continued to vary in some cases but seemingly bred true for resistance in others. Johnson concluded that inheritance for disease resistance in this case did not follow any simple Mendelian ratio but behaved in a manner which could be best explained by the multiple factor hypothesis.

Mackie (147) has recently studied the inheritance of resistance to rusty blotch (*Helminthosporium californicum* Mack and Pax.) in barley. In crosses between Abyssinian (susceptible) and Chevalier (immune) and the reciprocal, the F_1 plants were all immune, showing resistance to be dominant to susceptibility. In the F_2 generation the segregation occurred in the proportion of three non-attacked to one attacked, or in a 3:1 ratio, indicating a single factor difference for rusty-blotch resistance. F_3 families from the F_2 plants confirmed this ratio. No decided evidence of linkage was found.

Henry (109, 110) made crosses between varieties of flax to determine the inheritance of resistance to rust (*Melampsora lini*). Three immune varieties, Argentine Selection, Ottawa 770B, and Bombay, were crossed with susceptible ones and the F_1 plants of all crosses proved immune. Thus resistance proved to be a dominant factor. The immunity of Ottawa 770B and Bombay was in each case dependent upon a single dominant factor; whereas in Argentine Selection, apparently two dominant factors were present, either of which conditioned immunity. In crosses involving Ottawa 770B, rust reaction and flower color were inherited independently. These results were confirmed and extended by Myers (175) in 1937.

Mains (150) in 1931 reported some interesting results from his studies of the inheritance of resistance to leaf rust (*Puccinia sorghi*) of sweet corn. When selections of sweet corn highly resistant to physiologic forms 1 and 3 of the rust fungus were crossed with susceptible strains, the resistance was found to be inherited in a very definite manner, the segregation in the F_2 being three resis-

tant to one susceptible. When the F_1 of such crosses was back-crossed with the susceptible parents, the ratio obtained was one resistant to one susceptible. This indicated that the resistance of these selections to physiologic forms 1 and 3 of the rust fungus depended on a simple Mendelian factor. He studied two other selections that were resistant only to physiologic form 1 of the rust fungus. When these selections were crossed with susceptible selections, the resistance to physiologic form 1 was inherited in a ratio of three resistant to one susceptible. Back crosses of the F_1 by the susceptible parents gave a ratio of one resistant to one susceptible, indicating that their resistance to physiologic form 1 is likewise caused by a simple Mendelian factor. Mains found no indication of linkage of rust resistance with other factors.

Mains, Leighty, and Johnston (149) studied the inheritance of resistance to several physiologic forms of the leaf rust of wheat (*Puccinia triticina*) by crossing resistant and susceptible varieties. Resistance in the majority of these crosses proved to be a dominant factor. It was found that resistance was dependent on a single dominant genetic factor. Approximately three resistant to one susceptible segregate appeared in the F_2 generation. In the F_3 generation, the susceptible segregates bred true, while only one out of three of the resistant ones bred true, the other two segregating again into three resistant and one susceptible. These workers sum up the results of their studies with the statement: "Resistance to the various physiologic forms of leaf rust is due therefore to different factors, or groups of factors inherited as a unit, the different factors or groups being independently inherited. These may be brought together, thus uniting in a single strain the resistance to the various physiologic forms possessed by different varieties." These workers believe, therefore, that the resistance of any specific variety of wheat to a particular physiologic form of leaf rust is dependent upon a single independently inherited factor, or group of factors inherited as a unit.

Much work has been done on the inheritance of resistance to bunt, *Tilletia tritici* (Bjerk) Wint., in hybrids between susceptible and resistant varieties of wheat. Briggs (24–28) has reported interesting results in the inheritance of resistance to bunt in hybrids between susceptible varieties of wheat and the resistant varieties Martin, Hussar, White Odessa, and Banner Berkeley. Briggs

(24) presented data in 1926 that showed that the Martin variety differs from susceptible wheats, such as White Federation, in one main factor for resistance to bunt. Hussar wheat (24, 25) differs from such susceptible varieties in two factors for resistance to this disease. One is the same as the factor present in Martin, but the other allows bunt to develop on about half of the heterozygous plants. White Odessa wheat (26) has a single factor for resistance to bunt which is apparently identical with the Martin factor for resistances to bunt. In 1931, Briggs (27) presented data to show that Banner Berkeley differs from White Federation in one main factor for resistance to bunt and that this factor is the same as the one present in the Martin variety. Churchward (42) in 1931 reported that Florence differs from Hard Federation in one main factor for resistance to bunt. Briggs (28) in his later work with crosses of Florence × White Federation and Florence × Big Club secured data which he was unable to analyze satisfactorily for Mendelian ratios. He thought this might indicate the presence of a large number of modifying factors or possible multiple factors.

Gaines (79), in 1920, after studying the inheritance of resistance to bunt in wheat hybrids, concluded that bunt resistance in wheat is not a simple Mendelian unit character. He thinks that if bunt resistance is Mendelian it must be the result of multiple factors. Gaines (80) in 1923 in referring to bunt resistance in the wheat crosses of Turkey × Hybrid 128 and Fortyfold × Turkey states: "The picture of the inheritance in these two crosses is one in which susceptibility is dominant, with the susceptible segregates fluctuating around the mean of the susceptible parent in each case. It is difficult to place these phenomena of inheritance on a factorial basis on account of the seasonal fluctuations and the quantitative and comparative nature of the material. It is quite evident, nevertheless, that Turkey has several times as much resistance as Fortyfold."

Gaines (81, 82) in 1925 concludes that resistance to bunt in such varieties as Hussar, Martin, and White Odessa is probably due to a large number of unit factors, the cumulative effect of which is to make the total result appear dominant, and that a lesser number would give a recessive effect. He thinks the inheritance of resistance to bunt of wheat and covered smut of oats can best be interpreted on the basis of multiple factors. Wakabayashi (267) in

studying the inheritance of resistance to covered smut (*Ustilago levis*) in hybrid oats came to the conclusion that immunity was due to three independent dominant factors. Barney (15) analyzed three different typical crosses of oats in respect to their resistance and susceptibility to loose smut (*Ustilago avenae*) in which widely different ratios of resistance were obtained. He suggested the possibility that 1, 2, and 3 dominant factors for resistance would best explain the results.

The inheritance of resistance to stem rust (*Puccinia graminis* Pers.) of wheat has received much study in recent years. Clark (43) in studying the inheritance of resistance to this disease found that resistance in a cross between Kota and Hard Federation was recessive and occurred in the F_2 generation in about a 1:15 ratio. No F_3 family derived from these F_2 plants bred true. Aamodt (1) found that in a hybrid between Kanred and Marquis wheats the resistance of Kanred to physiologic form 1 of stem rust was inherited as a simple factor or as several linked factors with resistance dominant, a ratio of three resistant to one susceptible being produced in the F_2 generation. Segregates homozygous for resistance to this form were also resistant to several other physiologic forms to which Kanred is resistant. Melchers and Parker (163) obtained similar results with the same cross. Hayes and Aamodt (104) studied a cross between Kota and Marquis for resistance to two physiologic forms of stem rust. They found that resistance to both forms was combined in 3 out of 372 F_3 families. To form 27, Kota was immune and Marquis resistant. Besides homozygous immune and resistant families in the F_3 generation, families homozygous for susceptibility were obtained. Four types of heterozygous families were obtained. Immunity was dominant to both resistance and susceptibility. The results were explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility.

Puttick (203) found that in a cross between Mindum (a durum variety) and Marquis (a common wheat), reciprocally resistant and susceptible to physiologic forms 1 and 19 of stem rust, 35 F_2 plants out of a total of 388 combined the resistance of both parents. All gradations between complete susceptibility and immunity occurred.

It was considered that there is some evidence of a single main pair of genetic factors for the reaction to form 19. Harrington and Aamodt (98), in crosses between durum varieties, obtained somewhat similar results combining resistance to forms 1 and 34 of stem rust. Hayes, Parker, and Kurtzweil (103) found that resistance to a physiologic form of stem rust was partially dominant in an emmer-common cross, while it was recessive in a durum-common cross. There was some linkage of resistance with durum and emmer characters. Segregates were obtained which were even more resistant than the durum parent. Waldron (268) obtained somewhat similar results in a durum-common cross. However, the interpretation of the results is complicated by the sterility which occurs in such hybrids. Except in the last two investigations, no evidence of linkage of rust resistance was obtained.

Dietz (52) studied the inheritance of resistance to *Puccinia graminis avenae,* form 2, in oats. F_1, F_2, and F_3 generations of crosses between eight pure-line varieties of oats were artificially inoculated with stem rust in the greenhouse and field. Resistance was dominant and based on a single factor difference in the National × White Tartar and the Lincoln × White Tartar crosses; but in the crosses involving Burt varieties, at least two factors, one an inhibitor, were involved. Garber (83) reports the results of crossing two pure lines of oats, Minota and Victory, which are susceptible, with White Russian, which is relatively resistant to stem rust. A study of the F_2 and F_3 generations of these crosses indicated that resistance was dominant and that a single factor pair was responsible for the segregation observed, a ratio of three resistant to one susceptible being obtained in the F_2 generation. Parker (189) found that resistance to crown rust *Puccinia lolii avenae* (McAlpine) in oats (Burt × Sixty-Day) was inherited as a recessive and controlled by multiple factors.

Quisenberry (204) has recently studied the inheritance of resistance to stem rust in crosses between H–44, a spring wheat, and Minhardi, a winter wheat. A single-factor difference for resistance and susceptibility was indicated by the F_2 results. The F_3 studies, however, proved such an explanation to be inadequate. In addition to the major genetic factor involved, other minor modifying factors were assumed to explain the reaction obtained. Goulden, Neatby, and Welsh (95) in 1927 demonstrated that in the cross

H–44 × Marquis the inheritance of the high resistance of H–44 could be explained on the basis of a difference in the parents of a single main pair of factors. In the F_3 there was a 1:2:1 ratio of resistant, segregating, and susceptible lines respectively on the basis of rust reaction in the field. This segregation was shown to be independent of the seedling reaction to physiologic form 36 and a group of forms including form 21. This work was reported more fully in 1928 (96). The relatively simple inheritance of this type of mature plant resistance and the ease with which it may be used in plant breeding was made clear by Neatby and Goulden (178) in further studies involving a number of other crosses and by Clark and Ausemus (44) in 1928. Goulden (96) reported a similar condition in crosses involving Pentad as the resistant parent.

Wingard (287) in 1933 reported rust resistance in beans as a dominant factor. In the F_2 generation the segregation occurred in the proportion of three resistant plants to one susceptible, or in a 3:1 ratio, indicating a single factor difference for rust resistance. Powers and Hines (202) found the resistance of barley to two physiologic forms of *Puccinia graminis tritici* to be dominant to susceptibility, and to be differentiated by a single factor pair. This reaction to stem rust was not genetically linked with barbing of awns. Hayes *et al.* (105) studied the correlated reaction in several wheat crosses to stem rust, leaf rust, bunt, and black chaff and found the inheritance of stem-rust resistance of the mature-plant type of the H–44 parent to be dependent upon a single genetic factor difference. The moderate-plant resistance of two other strains of wheat appeared to be dependent upon factors not allelomorphic to those determining mature-plant resistance of the H–44 type. There was some indication that more than a single-factor pair was necessary to explain the stem-rust resistance of the mature plants of the H–35 parent in the crosses with Marquis. Brink and his associates (29) concluded that the resistance to bacterial wilt in alfalfa behaves in inheritance as an intergrading character and probably rests upon a complex genetic basis. They were unable to make a factorial interpretation of their results. Wellhausen (283) has found that at least three independently inherited dominant factors are involved in the inheritance of resistance to bacterial wilt in maize. Dundas (63), in studying the inheritance of resistance to powdery mildew in beans, concluded that pinto, the resistant

variety used, carries a single Mendelian factor pair for resistance to the strain of mildew employed. Emsweller and Jones (66) found that resistance to rust in the snapdragon is controlled by a single dominant gene. Their results, however, indicate the presence of modifying genes.

References

1. Aamodt, O. S. 1923. The inheritance of growth habit and resistance to stem rust in a cross between two varieties of common wheat. Jour. Agr. Res. **24**: 457–470.
2. Allen, Ruth F. 1923. A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici*. Jour. Agr. Res. **23**: 131–152.
3. ———. 1924. Cytological studies of infection of Baart, Kanred and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. Jour. Agr. Res. **26**: 571–604.
4. ———. 1926. A cytological study of *Puccinia triticina* physiologic form II on Little Club wheat. Jour. Agr. Res. **33**: 201–222.
5. ———. 1927. A cytological study of orange leaf rust, *Puccinia triticina* physiologic form II, on Malakoff wheat. Jour. Agr. Res. **34**: 697–714.

5*a*. ———. 1928. A cytological study of *Puccinia Glumarum* on *Bromus marginatus* and *Triticum vulgare*. Jour. Agr. Res. **36**: 487–513.

5*b*. ———. 1935. A cytological study of heterothallism in *Puccinia Sorghi*. Jour. Agr. Res. **49**: 1047–1068.

5*c*. ———. 1936. A cytological study of *Puccinia Malvacearum* from the sporidium to the teliospore. Jour. Agr. Res. **51**: 801–818.

6. Anderson, H. C. L. 1890. Rust in wheat. Agr. Gaz. N. S. Wales **1**: 81–92.
7. Anderson, P. J., Osmun, A. V., and Doran, W. L. 1926. Soil reaction and black root rot of tobacco. Mass. Agr. Exp. Sta. Bull. 229: 117–136.
8. ———. 1928. Soil reaction and black root rot of tobacco. (Abst.) Phytopath. **18**: 131.
9. ———, and Morgan, M. F. 1926. Black root rot and soil reaction. Conn. Agr. Exp. Sta. Tobacco Sta. Bull. 6: 59T–66T.
10. ———. 1929. Soil reaction studies on the Connecticut tobacco crop. Jour. Amer. Soc. Agron. **21**: 156–158.

10*a*. ———, Swanback, T. R., and Street, O. E. 1932. Tobacco substation at Windsor, report for 1931. Conn. Agr. Exp. Sta., New Haven, Bull. 335.

11. Armstrong, G. M., and Sumner, C. B. 1935. Investigation on downy mildew of tobacco. South Carolina Agr. Expt. Sta. Bull. 303.
12. Armstrong, S. F. 1922. The Mendelian inheritance of susceptibility and resistance to yellow rust (*Puccinia glumarum*) in wheat. Jour. Agr. Sci. **12**: 57–96.
13. Arthur, J. C., and Stuart, William. 1900. Corn smut. Ind. Agr. Exp. Sta. 12th Ann. Rep. 1898–1899. p. 84–135.
14. ———. 1903. Problems in the study of plant rust. Bull. Torrey Bot. Club **30**: 1–18.
15. Barney, A. F. 1924. The inheritance of smut resistance in crosses of certain varieties of oats. Jour. Amer. Soc. Agron. **16**: 283–291.
16. Barrus, M. F. 1921. Bean anthracnose. Cornell Univ. Agr. Exp. Sta. Mem. 42.

17. Bartholomew, L. K., and Jones, E. S. 1923. Relation of certain soil factors to the infection of oats by loose smut. Jour. Agr. Res. **24**: 569–575.
18. Bever, W. M. 1934. Effect of light on the development of the uredial stage of *Puccinia glumarum*. Phytopath. **24**: 507–516.
19. Biffen, R. H. 1905. Mendel's law of inheritance and wheat breeding. Jour. Agr. Sci. **1**: 4–48.
20. ———. 1907. Studies in the inheritance of disease resistance. Jour. Agr. Sci. **2**: 109–128.
21. ———. 1912. Studies in the inheritance of disease resistance. Jour. Agr. Sci. **4**: 421–429.
22. Bioletti, Frederic T. 1901. The Phylloxera of the vine. Calif. Agr. Exp. Sta. Bull. 131.
23. Brick, C. 1919. The resistance of certain varieties of our cultivated plants to parasites. Naturw. Wochenschr. **18**: 391–394.
24. Briggs, F. N. 1926. Inheritance of resistance to bunt, *Tilletia tritici* (Bjrek.) Winter, in wheat. Jour. Agr. Res. **32**: 973–990.
25. ———. 1930. Inheritance of the second factor for resistance to bunt, *Tilletia tritici*, in Hussar wheat. Jour. Agr. Res. **40**: 225–232.
26. ———. 1930. Inheritance of resistance to bunt, *Tilletia tritici*, in White Odessa wheat. Jour. Agr. Res. **40**: 353–359.
27. ———. 1931. Inheritance of resistance to bunt, *Tilletia tritici*, in hybrids of White Federation and Banner Berkeley wheats. Jour. Agr. Res. **42**: 307–313.
28. ———. 1932. Inheritance of resistance to bunt, *Tilletia tritici*, in hybrids of White Federation and Odessa wheat. Jour. Agr. Res. **45**: 501–505.
29. Brink, R. A., Jones, F. R., and Albrecht, H. R. 1934. Genetics of resistance to bacterial wilt in alfalfa. Jour. Agr. Res. **49**: 635–642.
30. Brown, B. E. 1930. Note regarding a possible influence of soil reaction on development of powdery mildew on cowpeas. Phytopath. **20**: 683–685.
31. Brown, William. 1936. The physiology of host-parasite relations. Bot. Rev. **2**: 236–281.
32. Bolley, H. L. 1889. Wheat rusts. Ind. Agr. Exp. Sta. Bull. 26.
33. Burkholder, W. H. 1918. The production of an anthracnose-resistant white marrow bean. Phytopath. **8**: 353–359.
34. Burnham, C. R. 1929. Studies on the inheritance of wilt resistance in flax. Doctor's thesis on the file of the University of Wisconsin library. (Original not seen.)
35. Butler, E. J. 1918. Immunity and disease in plants. Agr. Jour. India. (Special Indian Sci. Cong. Number) **13**: 10–82.
36. ———. 1918. Fungi and diseases in plants. 547 pp.
37. Carbone e Arnaudi. 1930. L'immunita nelle piante. Monografie dell'Institute Sieroterapico Milanese, 1930.
38. Chester, K. S. 1933. The problem of acquired physiological immunity in plants. Quart. Rev. Biol. **8**: 129–154, 275–324.
39. Christman, A. H. 1905. Observations on the wintering of grain rusts. Trans. Wis. Acad. Sci. **15**: 98.
40. Chupp, C. 1928. Club root in relation to soil alkalinity. Phytopath. **18**: 301–306.
41. ———. 1930. The effects of potash and phosphorus on the tip burn and mildew of cabbage. Phytopath. **20**: 307–318.
42. Churchward, J. G. 1931. Studies in the inheritance of resistance to bunt in a cross between Florence × Hard Federation wheats. Roy. Soc. N. S. Wales, Jour. and Proc. **64**: 298–319.
43. Clark, J. A. 1925. Segregation and correlated inheritance in crosses between Kota and Hard Federation wheats for rust and drought resistance. Jour. Agr. Res. **29**: 1–47.

44. Clark, J. Allen, and Ausemus, E. R. 1928. Immunity of Hope wheat from black stem rust inherited as a dominant factor. Jour. Amer. Soc. Agron. **20**: 152–159.
45. Clayton, E. E. 1936. Water soaking of leaves in relation to development of wildfire disease of tobacco. Jour. Agr. Res. **52**: 239–269.
46. ————. 1937. Water soaking of leaves in relation to development of blackfire disease of tobacco. Jour. Agr. Res. **55**: 883–890.
47. ————. 1923*a*. The relation of temperature to the Fusarium wilt of the tomato. Amer. Jour. Bot. **10**: 71–88.
48. Comes, Orazio. 1913. Della resistanza dei frumenti alle ruggini stato attuale della quistione e provvedimenti. Atti. R. Ist. d'Incor. Napoli, VI. **64**: 419–441. Letteratura e note, p. 437–440.
49. Conant, George H. 1927. Histological studies of resistance in tobacco to *Thielavia basicola*. Amer. Jour. Bot. **14**: 457–480.
49*a*. Cralley, E. M. 1939. Effects of fertilizer on stem rot of rice. Ark. Agr. Exp. Sta. Bull. 383.
50. Davis, R. J. 1925. Studies on *Ophiobolus graminis* Sacc., and the take-all disease of wheat. Jour. Agr. Res. **31**: 801–825.
51. DeBary, A. 1886. Ueber einige Sclerotinien und Sclerotienkrankheiten. Bot. Ztg. **44**: 377–387; 393–404; 409–426; 433–441; 449–461; 465–474.
52. Deitz, S. M. 1925. The inheritance of resistance to *Puccinia graminis avenae*. (Abst.) Phytopath. **15**: 54.
53. Delacroix, G. 1905. Observations et recherches au sujet de la "pourriture grise," etc. Bull. Mensuel de Renseign. Agricoles. April, 1905.
54. Dickson, J. G. 1923. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetti*. Jour. Agr. Res. **23**: 837–870.
55. ————, Eckerson, S. J., and Link, K. P. 1923. The nature of disease resistance to seedling blight of cereals. Proc. Nat. Acad. Sci. **9**: 434–439.
56. ————, and Holbert, J. R. 1928. The relation of temperature to the development of disease in plants. Amer. Nat. **62**: 311–333.
57. ————, Link, Karl P., and Dickson, A. D. 1933. Nature of resistance of corn to seedling blight. Abst. Phytopath. **23**: 9.
58. Dixon, Lawrence F., McLean, Ruth A., and Wolf, Frederick A. 1936. Relationship of climatological conditions to the tobacco Downy mildew. Phytopath. **26**: 735–759.
59. Doran, W. L. 1929. Effect of soil temperature and reaction on growth of tobacco infected and uninfected with black root rot. Jour. Agr. Res. **39**: 853–872.
60. ————. 1931. Increasing soil acidity as a means of controlling black root rot of tobacco. Mass. Agr. Exp. Sta. Bull. 276.
61. Duggar, B. M. 1909. Fungous diseases of plants. 508 pp.
62. ————. 1911. Physiological plant pathology. Phytopath. **1**: 71–78.
63. Dundas, B. 1936. Inheritance of resistance to powdery mildew in beans. Hilgardia **10**: 243–253.
64. Edgerton, C. W. 1910. The bean anthracnose. La. Agr. Exp. Sta. Bull. 119: 1–55.
65. ————, and Moreland, C. C. 1920. Tomato wilt. La. Agr. Exp. Sta. Bull. 174.
66. Emsweller, S. L., and Jones, H. A. 1934. The inheritance of resistance to rust in the snapdragon. Hilgardia **8**: 197–211.
67. Ericksson, J., and Henning, E. 1896. Die Getreideroste. 464 p.
68. Fahmy, T. 1931. The sore-shin disease and its control. Egypt Min. Agr. Plant Prot. Sect. Bull. 108.
69. Fehmi, Salahatten. 1933. Untersuchungen über den Einfluss der Ernährung auf die Empfänglichkeit der Kartoffelknolla für Lager-

parasiten und die Änderungen des enzymatischen Stoffwechselverlaufes während der Hagerung. Phytopath. Zeits. 6: 543–588.
70. Fellows, H. 1926. Relation of growth in the potato tuber to the potato scab disease. Jour. Agr. Res. 32: 757–781.
71. Finlow, R. S. 1918. Rhizoctonia in jute: the inhibiting effect of potash manuring. Agr. Jour. India 13: 65–72.
72. Foister, C. E. 1935. The relation of weather to fungous and bacterial diseases. Bot. Rev. 1: 497–516.
73. Freeman, E. M. 1911. Resistance and immunity in plant diseases. Phytopath. 1: 109–115.
74. ———, and Johnson, E. C. 1911. The rusts of grains in the United States. Bull. Bur. Plant Industry 216.
75. Fromme, F. D. 1913. The culture of cereal rusts in the greenhouse. Bull. Torrey Bot. Club 40: 501–521.
76. ———, and Wingard, S. A. 1918. Bean rust: Its control through the use of resistant varieties. Va. Exp. Sta. Bull. 220.
77. ———, and Murray, T. J. 1919. Angular leafspot of tobacco, an undescribed bacterial disease. Jour. Agr. Res. 16: 219–228.
78. ———, and Wingard, S. A. 1921. Varietal susceptibility of beans to rust. Jour. Agr. Res. 21: 385–404.
79. Gaines, E. F. 1920. The inheritance of resistance to bunt or stinking smut of wheat. Jour. Amer. Soc. Agron. 12: 124–132.
80. ———. 1923. Genetics of bunt resistance in wheat. Jour. Agr. Res. 23: 445–480.
81. ———. 1925. Inheritance of disease resistance in wheat and oats. (Abst.) Phytopath. 15: 51.
82. ———. 1925. The inheritance of disease of resistance in wheat and oats. Phytopath. 15: 341–349.
83. Garber, R. J. 1922. Inheritance and yield with particular reference to rust resistance and panicle type in oats. Minn. Agr. Exp. Sta. Tech. Bull. 7.
84. Gassner, Gustavo. 1909. Algunas Observaciones sobre el "Polvillo" de Los Porotos (*Uromyces appendiculatus*). Rev. Secc. Agron. Univ. Montevideo, No. 4, 1908. pp. 125–129.
85. Gassner, Gustav. 1915. Untersuchungen über die Abhänigigkeit des Auftretens der Getreideroste von auszeren Faktoren. Centbl. Bakt. 44: 512–617.
86. Gassner, G., and Straib, W. 1929. Untersuchungen über die Abhängigkeit des Infektionsverhaltens der Getreiderostpilze von Kohlensauregehalt der Luft. Phytopath. Zeits. 1: 1–30.
87. ———, and ———. 1931. Untersuchungen zur Frage der biologischen Spezializierung des Weizengelbrostes. Der Züchter 1931: 229–240.
88. ———, and Hassebrauk, K. 1931. Untersuchungen über die Beziehungen zwischen Mineralsalzernährung und Verhalten der Getreidepflanzen gegen Rost. Phytopath. Zeits. 3: 535–617.
89. ———, and Franke, W. 1934. Ueber den Einfluss der Temperatur auf Stickstoffgehalt und Rostresistenz junger Getreidepflanzen. Phytopath. Zeits. 7: 315–326.
90. Gaumann, E. 1932. Der Einfluss der Keimungstemperatur auf die chemische Zusammensetzung der Getreidekeimlinge. Zeits. Bot. 25: 385–461.
91. Gibson, C. M. 1904. Notes on infection experiments with various uredineae. New Phytol. 3: 184–191.
92. Gigante, G. 1935. Investigations of the influence of boron on the resistance of plants to parasitic attack. (trans. title.) (Bol. R. Staz. Patol. Veg. (Roma) n. ser. 15 (1935) No. 3, pp. 471–483, fig. 1: Eng. Abs. pp. 481–482.)

93. GILMAN, J. C. 1916. Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. **3**: 25–84.

94. GORDON, W. L. 1930. Effect of temperature on host reactions to physiologic forms of *Puccinia graminis avenae.* Erikss. and Henn. Sci. Agr. **11**: 95–103.

95. GOULDEN, C. H., NEATBY, K. W., AND WELSH, J. N. 1927. The inheritance of resistance to *Puccinia graminis tritici* in a cross between two varieties of *Triticum vulgare.* Anat. Rec. **37**: 2.

96. ————. 1928. The inheritance of resistance to *Puccinia graminis tritici* in a cross between two varieties of *Triticum vulgare.* Phytopath. **18**: 631–658. Illus.

97. ————. 1929. Breeding rust-resistant varieties of wheat—fundamental aspects of the problem. Sci. Agr. **10**: 258–267.

98. HARRINGTON, J. B., AND AAMODT, O. S. 1923. The mode of inheritance of resistance to *Puccinia graminis* with relation to seed color in crosses between varieties of Durum wheat. Jour. Agr. Res. **24**: 979–996.

99. HART, HELEN, AND FORBES, I. L. 1935. The effect of light on the initiation of rust infection. Phytopath. **25**: 715–725.

99*a*. ————, AND ZALESKI, KAROL. 1935. The effect of light intensity and temperature on infection of Hope wheat by *Puccinia graminis tritici.* Phytopath. **25**: 1041–1065.

100. HARTER, L. L., ANDRUS, C. F., AND ZAUMEYER, W. J. 1935. Studies on bean rust caused by *Uromyces phaseoli typica.* Jour. Agr. Res. **50**: 737–759.

101. HASKELL, R. J. 1919. Fusarium wilt of potato in the Hudson River Valley, New York. Phytopath. **9**: 223–260.

102. HASSEBRAUK, K. 1930. Relations of rust infection to mineral nutrition in cereals. Ang. Bot. **12**: 23–35.

103. HAYES, H. K., PARKER, J. H., AND KURTZWEIL, C. 1920. Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum.* Jour. Agr. Res. **19**: 523–542.

104. HAYES, H. K., AND AAMODT, O. S. 1923. A study of rust resistance in a cross between Marquis and Kota wheats. Jour. Agr. Res. **24**: 997–1012.

105. ————, AUSEMUS, E. R., STAKMAN, E. C., AND BEMBERG, R. H. 1934. Correlated inheritance of reaction to stem rust, leaf rust, bunt, and black chaff in spring wheat crosses. Jour. Agr. Res. **48**: 59–66.

106. HAZELWOOD, H. H. 1925. Rose mildew and potash. American Rose Annual.

107. HECKE, L. 1909. Der Einfluss von Sorte und Temperatur auf den Steinbrandbefall. Zeits. Landw. Versuchsw. Oesterr. **12**: 49–66.

108. ————. 1911. Beobachtungen der Überwinterungsart von Pflanzenparasiten. Naturw. Zeits. Landw. u. Fortsw. **9**: 44–53.

109. HENRY, A. W. 1926. Inheritance of immunity from *Melampsora lini.* (Abst.) Phytopath. **16**: 87.

110. ————. 1930. Inheritance of immunity from flax rust. Phytopath. **20**: 707–721.

111. HILTNER, L. 1914. Neuere Beobachtungen über den Rostbefall des Wintergetreides. Prakt. Bl. Pflanzenbau u. Pflanzenschutz, Jahrg. 17 (n. F. 12), p. 373–386.

112. HOFFER, G. N., AND FROST, J. F. 1925. Influence of balanced nutrient supply on susceptibility of corn plants to *Gibberella saubinetii* (Mont.) Sacc. Abs. in Phytopath. **15**: 59–60.

113. HOWITT, J. E. 1925. A review of our knowledge concerning immunity and resistance in plants. Quebec Soc. Prot. Plants Ann. Rept. **16**: 9–24.

114. HUMPHREY, H. B. 1914. Studies on the relation of certain species of

Fusarium to the tomato blight of the Pacific Northwest. Wash. Agr. Exp. Sta. Bull. 115.
115. HUNGERFORD, C. W. 1923. Studies on the life history of stripe rust, *Puccinia glumarum* (Schn.) Erikss. and Henn. Jour. Agr. Res. **24**: 607–624.
115a. HURSH, C. R. 1924. Morphological and physiological studies on the resistance of wheat to *Puccinia Graminis Tritici* Erikss. and Henn. Jour. Agr. Res. **27**: 381–411.
116. JARACH, MARCO. 1932. The mechanism of active acquired immunity in plants. Phytopath. Zeits. **4**: 315–327.
117. JOHANN, H., DICKSON, J. G., AND WINELAND, G. 1923. Relation of environment to infection of corn seedlings by *Diplodia zeae* (Schw.) Lev. (Abst.) Phytopath. **13**: 52.
118. JOHNSON, A. G. 1911. Further notes on timothy rust. Proc. Ind. Acad. Sci. **1910**: 203–204.
119. JOHNSON, J., AND HARTMAN, R. E. 1919. Influence of soil environment on the root-rot of tobacco. Jour. Agr. Res. **17**: 41–86.
120. ———. 1921. Inheritance of disease resistance to *Thielavia basicola*. (Abst.) Phytopath. **11**: 49.
121. ———. 1930. Breeding tobacco for resistance to *Thielavia* root rot. U. S. Dept. Agr. Tech. Bull. 175.
122. ———. 1937. Relation of water-soaked tissues to infection by *Bacterium angulatum* and *Bact. tabacum* and other organisms. Jour. Agr. Res. **55**: 599–618.
123. JOHNSTONE, K. H. 1931. Observations on the varietal resistance of the apple to scab (*Venturia inaequalis* Aderh.) with special reference to its physiological aspects. Jour. Pomol. & Hort. Sci. **9**: 195–227.
124. JOHNSON, T. 1931. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Dom. Canada Dept. Agr. Bull. 140.
125. JONES, E. S. 1923a. Influence of temperature, moisture, and oxygen on spore germination by *Ustilago avenae*. Jour. Agr. Res. **24**: 577–591.
126. JONES, L. R. 1905. Disease resistance of potato. U. S. Dept. Agr. Bur. Plant Ind. Bull. **87**: 14.
127. ———, AND GILMAN, J. C. 1915. The control of cabbage yellows through disease resistance. Wis. Agr. Exp. Sta. Res. Bull. 38.
128. ———. 1917. Soil temperatures as a factor in phytopathology. Plant World **20**: 229–237.
129. ———. 1921. Experimental work on the relation of soil temperatures to disease in plants. Trans. Wis. Acad. Sci. **20**: 433–459.
130. ———, MCKINNEY, H. H., AND FELLOWS, H. 1922. The influence of soil temperature on potato scab. Wis. Agr. Exp. Sta. Res. Bull. **53**: 1–35.
131. ———. 1923. Stem and root rot of peas in the United States Caused by species of Fusarium. Jour. Agr. Res. **26**: 459–476.
132. ———. 1924. The relation of environment of disease in plants. Amer. Jour. Bot. **11**: 601–609.
133. ———, AND LINFORD, M. B. 1925. Pea disease survey in Wisconsin. Wis. Agr. Expt. Sta. Res. Bull. 64.
133a. JONES, L. R., JOHNSON, JAMES, AND DICKSON, JAMES G. 1926. Wisconsin studies upon the relation of soil temperature to plant disease. Wis. Agr. Exp. Sta. Res. Bull. 71.
134. JORDI, ERNST. 1916. Über die Empfänglichkeit von *Phaseolus Vulgaris* L. für Bohnenrost. Zeits. Pflanzenkr. **26**: 374–375.
135. KINCAID, RANDALL R., AND GRATZ, L. O. 1935. Soil temperature studies on Florida cigar-wrapper tobacco. Jour. Agr. Res. **51**: 441–449.

136. KOLMER, JOHN A. 1917. A practical textbook on infection, immunity and specific therapy.
137. KOSTOFF, DONTCHO. 1934. Inheritance of natural immunity in plants with special reference to breeding of immune varieties. Zeits. Züchtung, Reihe A Pflanzenzüchtung **19**: 550–576.
138. KYLE, C. H. 1930. Relation between the vigor of the corn plant and its susceptibility to smut (*Ustilago zeae*). Jour. Agr. Res. **41**: 221–231.
139. LAURITZEN, J. I. 1919. The relation of temperature and humidity to infection by certain fungi. Phytopath. **9**: 7–35.
140. LEE, A. P., AND MARTIN, J. P. 1928. Fertilizer affects disease. Ind. & Eng. Chem. **20**: 2–20.
141. LEVINE, M. 1921. Studies on plant cancers. III. The nature of the soil as a determining factor in the health of the beet, *Beta vulgaris*, and its relation to the size and weight of the crown gall produced by inoculation with *Bacterium tumefaciens*. Amer. Jour. Bot. **8**: 507–525.
142. LINK, G. K. K. 1916. A physiological study of two strains of *Fusarium* in their causal relation to tuber rot and wilt of potato. Bot. Gaz. **62**: 169–209.
143. ———, AND WILCOX, H. W. 1936. Relation of nitrogen-carbohydrate nutrition of Stayman apple trees to susceptibility to fire blight. Phytopath. **26**: 643–655.
144. LITTLE, C. C., AND TYZZER, E. E. 1916. Further experimental studies on the inheritance of susceptibility to a transplantable tumor, carcinoma (J.W.A.) of the Japanese waltzing mouse. Jour. Med. Res. **33**: 393–453.
145. LITTLE, W. C. 1883. Report on wheat mildew. Jour. Roy. Agr. Soc. **19**: 643–691.
146. LUTMAN, B. F. 1934. Cell size and structure in plants as affected by various inorganic elements. Vt. Agr. Exp. Sta. Bull. 383.
147. MACKIE, W. W. 1928. Inheritance of resistance to rusty blotch in barley. Jour. Agr. Res. **36**: 965–975.
148. MAINES, E. B. 1917. The relation of some rusts to the physiology of their hosts. Amer. Jour. Bot. **4**: 179–220.
149. ———, LEIGHTY, C. E., AND JOHNSON, C. O. 1926. Inheritance of resistance to leaf rust, *Puccinia triticina* Erikss., in crosses of common wheat, *Triticum vulgare* VIII. Jour. Agr. Res. **32**: 931–972.
150. ———. 1931. Inheritance of resistance to rust, *Puccinia sorghi* Schw. in maize. Jour. Agr. Res. **43**: 419–430.
151. MARCHAL, E. 1901–03. Recherches de biologie experimentale appliqué à l'agriculture. I.
152. MCALPINE, D. 1906. The rusts of Australia. 349 p.
153. ———. 1911. Potato diseases in Australia.
154. MCCUE, C. A. 1913. Tomatoes for the canning factory. Del. Agr. Exp. Sta. Bull. 101: 18.
155. MCKINNEY, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Jour. Agr. Res. **26**: 195–217.
156. ———. 1925a. Preliminary environmental studies on the take-all disease of wheat caused by *Ophiobolus graminus* Sacc. Phytopath. **15**: 494–495.
157. ———. 1925b. Influence of soil temperature and moisture on infection of young wheat plants by *Ophiobolus graminus*. Jour. Agr. Res. **31**: 827–840.
158. MCNEW, G. L. 1937. Isolation of variants from cultures of *Phytomonas stewarti*. Phytopath. **27**: 1161–1170.
159. ———, AND SPENCER, ERNEST L. 1939. Effect of nitrogen supply of sweet corn on the wilt bacterium. Phytopath. **29**: 1051–1067.

160. McRostie, G. P. 1919. Inheritance of anthracnose-resistance as indicated by a cross between a resistant and a susceptible bean. Phytopath. 9: 141–148.
161. ———. 1921. Inheritance of disease resistance in the common bean. Jour. Amer. Soc. Agron. 13: 15–32.
162. Melander, L. W. 1935. Effect of temperature and light on development of the uredial stage of *Puccinia graminis*. Jour. Agr. Res. 50: 861–880.
163. Melchers, L. E., and Parker, J. H. 1922. Inheritance of resistance to black stem rust in crosses between varieties of common wheat (*Triticum vulgare*). (Abst.) Phytopath. 12: 31–32.
164. Melhardt, Helene. 1926. Die Abhängigkeit phanerogamet Parasiten von der Ernährung der Wirtspflanzen. Bot. Arch. 13: 447–474.
165. Melhus, I. E., Wilson, J. J., and Layton, Duke V. 1931. Three new wilt-resistant watermelons. Ia. Agr. Exp. Sta. Circ. 125.
166. ———, and Kent, George C. 1939. Elements of plant pathology.
167. Metha, K. C. 1923. Observations and experiments on cereal rusts in the neighborhood of Cambridge, with special reference to their annual recurrence. Trans. Brit. Mycol. Soc. 8: 142–176.
168. Milbrath, D. C. 1918. (Field Notes). U. S. Dept. Agr. Bur. Pl. Ind. Off. Cotton, Truck, Forage Crop Dis. Invest. News Notes 7 (22): 5.
169. Miles, L. E. 1936. Effect of potash fertilizers on cotton wilt. Miss. Agr. Expt. Sta. Tech. Bull. 23.
170. Millikan, C. R. 1939. The influence of nutrition on the reaction of wheat to *Urocystis tritici Koern*, III. Jour. Dept. Agr. Victoria 37: 587–596.
171. Miyake, K., and Adachi, M. 1922. Chemische Untersuchungen über die Widerstandsfähigkeit der Reisarten gegen die "Imochi-Krankheit" (Erster Bericht). Jour. Biochem. Tokyo. 5: 223–239.
172. Montemartini, L. 1909. LaRuggine die cereali in raporto colla concimazione. Riv. Pathol. Veg. Ann. 4: 53–56.
173. Moss, E. G., McMurtrey, J. E., Lunn, W. M., and Carr, J. M. 1927. Fertilizer tests with flue-cured tobacco. U. S. Dept. Agr. Tech. Bull. 12.
174. Muller, H. C., and Molz, Emil. 1917. Über das Auftreten des Gelbrostes (*Puccinia glumarum*) am Weizen in den Jahren 1914 und 1916. Fuhl. Landw. Ztg., 66: 42–55.
175. Myers, W. M. 1937. The nature and interaction of genes conditioning reaction to rust in flax. Jour. Agr. Res. 55: 631–666.
176. Nakata, K. 1934. Sukumotsu Byogai Zuhen (illustrated crop diseases.) Tokyo.
177. Neal, David C. 1927. Cotton Wilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. 14: 359–424.
178. Neatby, K. W., and Goulden, C. H. 1930. The inheritance of resistance to *Puccinia graminis tritici* in crosses between varieties of *Triticum vulgare*. Sci. Agr. 10: 389–404.
179. Newton, Margaret T., Johnson, T., and Brown, A. M. 1933. Stripe rust in Canada. (Abst.) Phytopath. 23: 25–26.
180. Nightengale, Alice A. 1936. Some chemical constituents of apple associated with susceptibility to fire blight. N. J. Agr. Exp. Sta. Bull. 613.
181. Nilsson-Ehle, H. 1911. Resistenz gegen Gelbrost beim Weisen. Kreuzungsuntersuchungen an Hafer und Weisen. II. Lund's Univ. Arsskr. 7: 57–82.
182. Norton, J. B. S. 1916. Internal action of chemicals on resistance of tomatoes to leaf disease. Md. Agr. Exp. Sta. Bull. 192: 17–30. illus.

183. ORTON, W. A. 1902. On the breeding of disease resistant varieties. Proc. Inter. Conf. Pl. Breed. Hybrid. (In Hort. Soc. N. Y. Mem. 1: 41–54. 1902.)
184. ———. 1907. A study of disease resistance in watermelons. Science 25: 288.
185. ———. 1908. On the theory and practice of breeding disease-resistant plants. Amer. Breeders' Assoc. Ann. Rept. 4: 145–156.
186. ———. 1909. The development of farm crops resistant to disease. U. S. Dept. Agr. Yearbook 1908: 453–464.
187. ———. 1911. The development of disease resistant varieties of plants. IV. Conf. Inter. Genet., Paris. Comptes Rendes et Rapports, 247–265.
188. ———. 1914. Potato wilt, leaf-roll, and related diseases. U. S. Dept. Agr. Bull. 64.
189. PARKER, J. H. 1920. A preliminary study of the inheritance of rust resistance in oats. Jour. Amer. Soc. Agron. 12: 23–38.
190. PEACOCK, R. W. 1911. Rust in wheat and oats. Agr. Gaz. N. S. Wales 22: 1013–1016.
191. PELTIER, G. L. 1919. Snapdragon rust. Ill. Agr. Exp. Sta. Bull. 221.
191*a*. PELTIER, GEORGE L. 1918. Susceptibility and resistance to citrus-canker of the wild relatives, citrus fruits, and hybrids of the genus *Citrus*. Jour. Agr. Res. 14: 337–358.
192. ———. 1923. A study of the environmental conditions influencing the development of stem rust in the absence of an alternate host. II. Infection studies with *Puccinia graminis tritici* form 3 and form 9. Nebr. Agr. Exp. Sta. Bull. 25.
193. ———. 1925. VI. Influence of light on infection and subsequent development of urediniospores of *Puccinia graminis tritici* on wheat. Nebr. Agr. Exp. Sta. Bull. 35.
194. PETERMANN, A. 1902. Rapport sur les travaux de 1901. Bull. Inst. Chim. et. Bact. à Gembloux. No. 72.
195. PETRI, L. 1930. Relation of mineral nutrients of plants to predisposition or resistance to pathogenic agencies. Boll. R. Staz. Patol. Veg. 10: 121–152.
196. PETURSON, B. 1930. Effect of temperature on host reactions to physiologic forms of *Puccinia coronata avenae*. Sci. Agr. 11: 104–110.
197. PIEMEISEL, F. J. 1917. Factors affecting the parasitism of *Ustilago zeae*. Phytopath. 7: 294–307.
198. PORTER, D. R. 1926. Watermelons that won't wilt on sick ground. Ia. Hort. Soc. Trans. 61: 213–216.
199. ———. 1928. Infection studies with watermelon wilt caused by *Fusarium niveum* EFS. Ia. Agr. Exp. Sta. Res. Bull. 112.
200. ———, AND HENDERSON, W. J. 1928. Progress report on vegetable disease investigations in Iowa, 1928. Ia. Hort. Soc. Trans. 63: 234–241.
201. ———, AND MELHUS, I. E. 1932. The pathogenicity of *Fusarium niveum* (EFS) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad.). Ia. Agr. Exp. Sta. Res. Bull. 149.
202. POWERS, LEROY, AND HINES, LEE. 1933. Inheritance of reaction to stem rust and barbing of awns in barley crosses. Jour. Agr. Res. 46: 1121–1129.
203. PUTTICK, G. F. 1921. The reaction of the F_2 generation of a cross between a common and a durum wheat to two biologic forms of *Puccinia graminis*. Phytopath. 11: 205–213.
204. QUISENBERRY, KARL S. 1931. Inheritance of winter hardiness, growth habit, and stem-rust reaction in crosses between Minhardi winter and H–44 spring wheats. U. S. Dept. Agr. Tech. Bull. 218.
205. RAINES, M. A. 1922. Vegetative vigor of the host as a factor influenc-

ing susceptibility and resistance to certain rust diseases of the higher plants. Amer. Jour. Bot. **9**: 215–238.

206. Ramsbottom, J. 1914–15. Iris leaf blotch. Jour. Roy. Hort. Soc. **40**:
207. Reddy, C. S. 1933. Resistance of dent corn to *Basisporium gallarum* Moll. Ia. Agr. Exp. Sta. Res. Bull. 167.
208. Reimer, F. C. 1925. Blight resistance in pears and characteristics of pear species and stocks. Ore. Agr. Exp. Sta. Bull. 214.
209. Remer, W. 1904. Der Rost des Getreides in Schlesien im Sommer. 1903. Zeits. Pflanzenkr. **14**: 63–70.
210. Reyes, G. M. 1929. A preliminary report on the stem rot of rice. Philippine Agr. Rev. **22**: 313–331.
211. Rice, M. A. 1927. The haustoria of certain rusts and the relation between the host and pathogene. Bull. Torrey Bot. Club **54**: 63–154.
212. ———. 1935. The cytology of host-parasite relations. Bot. Rev. **1**: 327–353.
213. Richards, B. L. 1923a. Further studies on the pathogenicity of *Corticium vagum* on the potato as affected by soil temperature. Jour. Agr. Res. **23**: 761–770.
214. ———. 1921. Pathogenicity of *Corticium vagum* on the potato as affected by soil temperature. Jour. Agr. Res. **21**: 459–482.
215. Rivera, V. 1913. A preliminary contribution on the receptivity of oak for Oidium. Atti. R. Acad. Lincei, Rend. CL. Sc. Fis. Mat. e Nat. V. **22**: 168–173.
216. ———. 1924. Cryptogamic epidemics and the environmental factors that determine them. Int. Rev. Sci. Pract. Agr. N. S. **2**: 604–610.
217. Rolfs, F. M. 1915. Angular leaf spot of cotton. S. C. Agr. Exp. Sta. Bull. 184.
218. ———. 1915. A bacterial disease of stone fruits. Cornell Univ. Agr. Sta. Mem. 8.
219. Russell, E. J. 1918. Soil conditions and plant growth.
220. ———. 1919. A student's book on soils and manures.
221. Sakurai, M. 1917. On the sclerotial disease of rice. Ehime Agr. Exp. Sta. Pub. No. 1.
222. Salmon, E. S. 1905. On the stages of development reached by certain biologic forms of Erysiphe in cases of non-infection. New Phytol. **4**: 217–222.
223. ———. 1914. American gooseberry mildew. Jour. Board Agr. 20. 1913–1914.
224. Sanford, G. B. 1926. Some factors affecting the pathogenicity of *Actinomyces scabies*. Phytopath. **16**: 525–547.
225. Schindler, Franz. 1909. Der Getreidebau auf wissenschaftlicher und praktischer Grundlage.
226. Selby, A. D., and Hickman, J. F. 1897. Corn smut. Ohio Agr. Exp. Sta. Bull. 78.
227. Sempio, Cesare. 1938. Influenza Della Luce E Dell' Oscurita Sui Principali Periodi Del Parassitamento. Rivista di Patologia Vegetale **19**: 1–73.
228. Sharvelle, E. G. 1936. The nature of resistance of flax to *Melamposora lini*. Jour. Agr. Res. **53**: 81–127.
229. Shaw, Luther. 1934. Studies on resistance of apple and other rosaceous plants to fire blight. Jour. Agr. Res. **49**: 283–313.
230. ———. 1935. Intercellular humidity in relation to fire-blight susceptibility in apple and pear. Cornell Univ. Agr. Exp. Sta. Mem. 181.
231. Sheldon, J. L. 1903. Preliminary studies on the rusts of the asparagus and carnation. Science **16**: 235–237.
232. ———. 1905. The effect of different soils on the development of the carnation rust. Bot. Gaz. **40**: 225–229.

233. SHERWOOD, E. C. 1923. Hydrogen-ion concentration as related to the Fusarium wilt of tomato seedlings. Amer. Jour. Bot. **10**: 537–553.
234. SMITH, E. F. 1911. Bacteria in relation to plant diseases. Carnegie Inst. Wash. Pub. 27.
235. SMITH, RALPH E. 1914. Plant Pathology. Cal. Agr. Exp. Sta. Rep. 1913–14.
236. SPENCER, ERNEST L., AND MCNEW, GEORGE L. 1938. The influence of mineral nutrition on the reaction of sweet-corn seedlings to *Phytomonas stewarti*. Phytopath. **28**: 213–223.
237. SPINKS, G. T. 1913. Factors affecting susceptibility to disease in plants. Jour. Agr. Sci. **5**: 231–247.
238. STAKMAN, E. C. 1914. A study in cereal rusts. Minn. Agr. Exp. Sta. Bull. 138.
239. ———, AND LEVINE, M. N. 1919. Effect of certain ecological factors on the morphology of the urediniospores of *Puccinia graminis*. Jour. Agr. Res. **16**: 43–77.
240. ———, AND AAMODT, O. S. 1924. The effect of fertilizers on the development of stem rust of wheat. Jour. Agr. Res. **27**: 341–380.
241. STEWART, J. P. 1912. Factors influencing yield, color, size, and growth in apples. Relation of fertilization to fire blight. Rep. Penn. Agr. Exp. Sta. 1910–1911: 467.
242. STEWART, V. B. 1913. The fire blight disease in nursery stock. Cornell Univ. Agr. Exp. Sta. Bull. 329.
243. STRANAK, F. 1915. Zur Frage der Bekämpfung des Gelbrostes. Deut. Landw. Presse, **42**: 379.
244. TAUBENHAUS, J. J., EZEKIEL, W. N., AND KILLOUGH, D. T. 1928. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bull. 389: 1–19.
245. TAYLOR, HENRY M. 1928. Virginia Farm Statistics—1927. Va. Dept. Agr. Bull. 5.
246. TEHON, L. R., AND YOUNG, P. A. 1924. Notes on the climatic conditions influencing the 1923 epidemic of stem rust on wheat in Illinois. Phytopath. **14**: 94–100.
247. THOMAS, H. E. 1921. The relation of the health of the host and other factors to infection of *Apium graveolens* by *Septoria apii*. Bull. Torrey Bot. Club **48**: 1–29.
248. THOMAS, H. E., AND MULLER, ALBERT S. 1929. Some factors which influence the infection of *Apium graveolens* L. by *Septoria apii* rostr. Amer. Jour. Bot. **16**: 789–798.
249. ———, AND ARK, P. A. 1934. Fire blight of pears and related plants. Cal. Agr. Exp. Sta. Berkeley Bull. 586.
250. TIMS, E. C. 1926. The influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. Jour. Agr. Res. **33**: 971–972.
251. TISDALE, W. H. 1917. Flax wilt: A study of the nature and inheritance of wilt resistance. Jour. Agr. Res. **11**: 573–606.
252. TISDALE, W. B. 1917a. Relation of temperature to the growth and infecting power of *Fusarium lini*. Phytopath. **7**: 356–360.
253. ———. 1923. Influence of soil temperature and soil moisture upon the *Fusarium* disease in cabbage seedlings. Jour. Agr. Res. **24**: 55–86.
254. ———, AND KELLEY, J. G. 1926. A Phytophthora Disease of Tobacco. Fla. Agr. Exp. Sta. Bull. 179: 159–218.
255. TORSELL, ROB. 1918. Iakttagelser Rorande Den S. K. Slidsjukans Upptradande. A hostvete vid ultuna Sommaren 1918. Sveriges Utsadesfor. Tidskr., Agr. 28, Hafte 6, pp. 260–274. Abstract in Bot. Abs. **6**: 104. 1920.
256. TRELEASE, S. F., AND TRELEASE, H. M. 1928. Susceptibility of wheat

to mildew as influenced by salt nutrition. Bull. Torrey Bot. Club. 55: 41–68.

257. Tucker, C. M. 1931. Taxonomy of the genus phytophthora De Bary. Mo. Agr. Exp. Sta. Res. Bull. 153.
258. Turner, T. W. 1922. Studies of the mechanism of the physiological effects of mineral salts in altering the ratio of top to root growth in seed plants. Amer. Jour. Bot. 8: 415–445.
259. Valleau, W. D. 1928. *Bacterium pruni* problems in Kentucky. U.S.D.A. Pl. Dis. Rep. 22: (9).
260. Vanterpool, T. C. 1935. Studies on browning root rot of cereals. III. Phosphorus-nitrogen relations of infested fields. IV. Effects of fertilizer amendments. V. Preliminary plant analyses. Canad. Jour. Res. 13: 220–250.
261. Vavilov, N. I. 1913. Beitrage zur Frage über die verschiedene Widerstandsfahigkeit der Getreide gegen parasitische Pilze. Trudy Selek. Stan. Moskov. Selskokhoz. Inst. 1: p. 1–108.
262. ————. 1914. Breeding for resistance to rust and mildew in wheat.
263. ————. 1914. Immunity to fungous diseases as a physiological test in genetics and systematics exemplified in cereals. Jour. Genet. 4: 49–63.
264. ————. 1919. Immunity of plants to infectious diseases. Proc. Petrovski Agr. Acad. 1918: 1–239.
265. Voelcker, J. A. 1912. The Woburn pot-culture experiments, 1910–1911–1912. Jour. Roy. Agr. Soc. England 73: 314–338.
266. Wade, B. L. 1929. Inheritance of Fusarium wilt resistance in canning peas. Wis. Agr. Exp. Sta. Res. Bull. 97.
267. Wakabayashi, S. 1921. A study of hybrid oats, *Avena sterilis* × *Avena orientalis*. Jour. Amer. Soc. Agron. 13: 259–266.
268. Waldron, L. R. 1921. Inheritance of rust resistance in a family derived from a cross between durum and common wheat. N. D. Agr. Exp. Sta. Bull. 147.
269. Walker, J. C. 1921. Relation of soil temperature and other factors to onion smut infection. Jour. Agr. Res. 22: 235–262.
270. ————. 1924. On the nature of disease resistance in plants. Trans. Wis. Acad. Sci. 21: 225–247.
271. ————. 1926. The influence of soil temperature and soil moistures upon white-rot of *Allium*. Phytopath. 16: 697–710.
272. ————. 1926. Studies upon the inheritance of Fusarium-resistance in cabbage. (Abst.) Phytopath. 16: 87.
273. ————. 1930. Inheritance of Fusarium-resistance in cabbage. Jour. Agr. Res. 40: 721–745.
274. Ward, H. M. 1902. Experiments on the effect of mineral starvation on the parasitism of the uridine fungus, *Puccinia dispersa*, on species of *Bromes*. Proc. Roy. Soc. London 71: 138–151.
275. ————. 1902. On the relations between host and parasite in the bromes and their brown rust, *Puccinia dispersa* (Erikss.) Ann. Bot. 16: 233–315.
276. ————. 1905. Recent researches on the parasitism of fungi. Ann. Bot. 19: 1–54.
277. Ware, J. O., and Young, V. H. 1934. Control of cotton wilt and "rust." Ark. Agr. Exp. Sta. Bull. 308.
278. Waterhouse, W. L. 1929. Australian rust studies I. Proc. Linn. Soc. N.S.W. 54: 615–680.
279. Waters, Charles W. 1928. The control of teliospore and urediniospore formation by experimental methods. Phytopath. 18: 157–213.
280. Wei, C. T. 1937. Rust resistance in garden bean. Phytopath. 27: 1090–1105.

experimentally induced wound healing may be justly called a study of cellular reactivity or competence in relation to plant organization, and it would appear that such studies should be of considerable advantage in our attempt to gain an understanding of the developmental basis of plant structure.

Under comparable conditions of growth, one and the same plant type usually exhibits some uniformity of reaction, but considerable differences occur between various plant types. Of particular interest are certain tissue changes resembling wound reactions which occur in apparently intact organs. Examples of these are the early stages of normal cork formation and certain intumescences.

Well known types of wound healing are the following examples: (1) In the terminal growing regions of shoot and root, the outermost, completely undifferentiated parts of the indeterminate meristems may reproduce parts lost by injury completely and more or less directly from cells abutting on the cut surface. (2) Further distant from the apex the responses become more complicated. Wound repair here is often effected by "secondary" meristems, *e.g.*, through a cork phellogen or through cambia and calluses from primary or secondary tissues. Original differentiation is influenced in various ways by both inner and outer conditions, and may become inhibited as well as accelerated. (3) In plants or organs of low reactivity or in maturated zones, cells of the ground tissue may not divide at all. It has been found, however, that in many cases redifferentiation, such as specialized thickening and chemical changes in the wall, may still be induced, and the tissue pattern near the injury may thus become partly restored.

The formation of the meristem is usually accompanied by some degree of differentiation, especially in the wall. More frequently specific cell and tissue types are produced. This adaptation of differentiating or mature tissues to the new conditions created by the injury in a fashion of controlled rejuvenation and redifferentiation is a characteristic feature of wound healing and cellular behavior in higher plants. This behavior would seem to explain why generally in plants cell and tissue forms of reparation tissues do not differ much from those found normally; they are as variable in degree and quality as the mother structures on which they arise, giving evidence of the developmental potencies of these at the time when the injury occurred.

Experimentally induced wound healing is a valuable tool in the analysis of the cytological, histological, and physiological sides of tissue development and the causes and mechanics of cell division and differentiation. By this method it is possible to reproduce, under controlled conditions, tissue structures within limited areas, and to investigate cell division and growth conveniently in large, more or less vacuolate cells. Though only in its beginning, this method has already yielded tangible results and thrown light on fundamental processes, such as the changes which precede the rejuvenation of cells, the mechanism and plane of nuclear and cell division, and the problem of subsequent controlled redifferentiation.

ORGAN STRUCTURE AND WOUND RESPONSE

Stems

Following Mohl's (130) early and exact description of periderm formation in natural and artificial wounds, numerous attempts have been made to describe and analyze the sequence of anatomical and physiological changes after wounding. Storage organs, such as potato tubers and kohlrabi stems, are suitable material for the study of the wide variety of phenomena connected with phellogen formation and structural regeneration. Here the processes during the formation of the temporary barrier of collapsing and suberizing cells, the metabolic changes during subsequent meristem formation, and the influence of lepto- and wound hormones have been extensively studied. External conditions (humidity, light, temperature, access of oxygen) influenced the traumatic response both qualitatively and quantitatively, as was also evident from various wound treatments which affected transpiration or facilitated or reduced the exchange of gases.

It is now generally agreed that certain metabolic changes in the cells abutting on the cut surface (enzyme activation, increase in the rate of cytoplasmic streaming, transformation of carbohydrates and nitrogenous substances, and changes in acidity) are accompanied by increased oxygen absorption and carbon dioxide production, and that access of oxygen is essential in this process.

Furthermore, changes of a necrobiotic type usually occur in a number of cells which have suffered irreversible changes in permeability, coagulation of protoplasm, and infiltration of the intercellular spaces with sap. Cells in such regions may subsequently degenerate

and die, and their contents and membranes may undergo further postmortal changes which probably affect the metabolism of adjacent healthy cells.

It has been found that satisfactory suberization and wound cork formation in the potato proceed best under relatively high humidity and temperature conditions (4, 196, 187); exposure of wounded parts also would appear to facilitate the exchange of gases and respiration (87).

Heat production in wounded parts of plants has been reported; in potato such an inflammatory condition reaches its maximum soon after wounding, decreasing with the distance from the injury, and reaching the norm again after about two days (43).

Excessive humidity creates a hyperhydric condition in plant cells, promoting cell proliferation but reducing cell division and differentiation. Periderm formation in submerged dicotyledonous plants, however, is possible (151).

Haberlandt used the technique of washing the injured surfaces which results in reduced cell division and metabolic activity in cells adjacent to the layer of crushed and degenerative cells. The interpretation of this effect, however, is contradictory. Lutman (122) emphasized that washing may remove the wound substances from the surface as well as other essential substances contained within the cells; but one important result was greatly reduced wound respiration. Steward, Wright and Berry (170) studied this effect in potato discs placed in air or water, or placed in air after preliminary washing; they found the amount of respiration related to the number of air spaces infiltrated with water. If all air spaces are filled, only little oxygen has access to the tissue. It appears that the result of an infiltration with tissue sap instead of water is rather different, for we know that in this case numerous cell divisions occur. This is in agreement with Ruhland and Ramshorn's findings (153), who reported that the application of tissue sap increased respiration greatly; they attributed this phenomenon to the presence in the juice of wound hormones or growth factors which regulate respiration.

It is natural that during progressive suberization and periderm formation the entry of oxygen should become considerably impeded. Thornton (175) found that oxygen regulates the dormancy of the potato in such a manner that during the progress of suberization

the entry of oxygen into the tuber is actually retarded, while the sprouting of the buds is promoted.

Much early work was done on wound repair and regeneration in terminal growing points, but some more recent reports on wound healing of apices have been somewhat contradictory. Mirskaja (129) described a case of regeneration of the terminal stem meristem of *Tradescantia guianensis* which was comparable to the well known method of direct regeneration in wounded root tips. Linsbauer (119) and also Pilkington (143) reinvestigated these processes in herbaceous monocotyledons and dicotyledons, and confirmed the former author's earlier findings, that only a limited portion of the apex above and between the youngest leaf primordia is capable of reproducing the terminal meristem; generally new growing points are formed from parts of the original meristem which have remained intact.

Bloch (17) investigated cell reactivity in the primary stem tissues of a monocotyledon, *Tradescantia fluminensis*. All living vacuolate cells, including epidermis, collenchyma and sclerenchyma, were capable of returning to a meristematic condition and of forming storied cork, though to a lesser degree in the older internodes. Formation of calcium oxalate crystals, not in the form of normal monoclinic raphides, but as tetragonal trihydrate, was combined with the resumption of meristematic activity. Cells surrounding the internal vascular bundles, normally richer in cytoplasm than the adjacent parenchyma, differentiated into thick-walled lignified elements.

The wound tissues of woody stems are known for their extensive dimensions, the high degree of differentiation, and the frequent irregularity of elements produced. Both cambium and primary and secondary tissues take part in their formation. Methods of callus formation in grafts were reported by Sass (155) and Sharples and Gunnery (163); the former author found that in apple grafts the callus was derived from all the living tissues located outside the xylem, the cambium itself contributing very little; the latter authors described the method of callus formation in cleft grafts and stripped surfaces of *Hibiscus* and *Hevea;* here callus was formed mainly from the medullary rays. Mendel (126) studied callus and wound gum formation in *Citrus* grafts.

In practical tree surgery best results were obtained with vigorous

specimens and when operations were carried out during spring or early summer (171, 124, 36). Wray (195) and also Paterson (141) investigated the structural changes which occur after pruning. The latter author, discussing the advantages of live-pruning in *Picea excelsa,* reported that live-pruned branches occluded more rapidly causing less distortion of the grain than dead-pruned ones.

A. B. Brown (28) studied cambial activity in relation to wounding in *Populus balsamifera;* the presence of living bark, developing buds and leaves distal to the wound had a promoting influence on local wound cambial activity. This responds to the stimulus of gravity in the same way as normal cambial activity, and the author infers that hormone action is here involved. Besides this, another local stimulating effect on cambial activity was exerted by dying cells of the cortex, where wound substances might be active.

Willison (191) reported fall spray injuries in peach trees that were due to treatment with 1:7 lime sulphur mixture, which prevented the periderm from developing over the leaf scars.

Poulter (144) investigated wound healing in alfalfa, white clover and red clover stems in relation to cutting and pasturing. In red clover the amount of dying pith cells is large and wound cork formation very slow, so that late autumn injuries do not properly heal.

Roots

Roots, both normal and air roots, particularly of monocotyledons, furnish excellent examples of different types of wound responses (14, 15, 16, 19). Generally there seems to exist a correlation between normal phellogen formation and the capacity to form wound cork. In extreme cases, however, neither is formed. In such a case the response to wounding may be simple suberization or metacuticularization of the ground parenchyma cells abutting on the wound (*Allium cepa*), or cell expansion and considerable structural redifferentiation may occur (air roots of orchids). In the cortex and axial strand even heavily impregnated cells may dedifferentiate (air roots of *Araceae* and orchids). A well developed endodermis, however, usually acts as a barrier preventing the wound stimulus from passing over to the axial strand. Deep cortex wounds accelerate the differentiation of endodermal cells and may promote activity in the pericycle, even when the cortex cells themselves are incapable of dividing (air roots of orchids).

Wounding of root tips of *Faba vulgaris, Lupinus* sp. and other plants has, according to Fourcroy (55, 56), an inhibiting effect on the differentiation of the elements determined to be the future first vessels, but it accelerates the development of the ontogenetically later elements which are not directly affected by the injury.

Taubenhaus and Ezekiel (174) described pathological enlargement and wounds in tap roots of cotton caused by acid soil (pH 2–4). Gore and Taubenhaus (65) studied the proliferation thus induced in cambium, phellogen and ray cells.

Cotyledons

Distribution and plane of cell division in cotyledons in relation to a wound surface and to the presence of vascular elements have been studied by various workers. Nakano (134), using cotyledons of *Vicia, Phaseolus, Pisum, Lupinus* and *Cucurbita,* assumed the combined action of an irritant diffusing from the wound surface (wound hormone) and a factor from the vascular bundle, probably the leptom (leptohormone). In this case the assumption was based particularly on the observation (also often reported in the older literature) that parenchyma cells situated between a vascular bundle and the wound face divide more vigorously. Badian (7), in a study of cotyledons of *Lupinus* and *Cucurbita,* confirmed these results; he also paid special attention to later phases of divisions at the cut ends of vascular bundles, whose plane is probably no longer determined by the traumatic stimulus.

In this connection it is of interest that cases have been reported in which an influence of the leptom on cell division appears unlikely, for instance, in the endosperm of *Ricinus* (134) and *Crinum asiaticum* (127).

Leaves

Woit (193) studied wound healing, cell reactivity and intumescence formation in tunnels of leaf miners in relation to leaf age; thin leaves of annuals tended to form callus, fleshy leaves and leaves of evergreens periderm. Most cell types were capable of renewed growth and division; the former was promoted when transpiration was reduced.

Wylie (197, 198) reported that the formation of a protective barrier consisting of collapsing dead cells generally preceded the formation of the meristem. In leaves of evergreens callus and

wound cork formation proceeded much more slowly than in mesophytic leaves, but larger amounts were formed. In leaves of *Syringa vulgaris* (199), wounds were made which interrupted the major conductive channels; these injuries healed, but there was no formation of new vascular bridges as reported in other instances. Here the sap supply to the partly isolated portions of the blade was apparently transported through the few remaining small veins.

Jump (92) studied wound healing in leaves of *Ficus australis* and found that besides the formation of periderm, the mesophyll often proliferated into the gap, finally effecting a union of the wound surfaces.

Bloch (18, 19) investigated the histological and microchemical changes in the natural wounds of the stem-borne leaves of *Araucaria imbricata*. Longitudinal fissures appear in the 5–40-year-old leaves owing to radial expansion of the axis. A many-layered periderm, tyloses and wound gum are formed, and the membranes of lignified fibres adjacent to the wounds undergo postmortally a very gradual enzymatic breakdown.

Fritz (58) wounded the outer wall of epidermal cells of *Gasteria*, and observed that the outer layer rich in cutin became readily regenerated.

LaRue (111, 112, 113, 114) examined the structure and physiology of intumescences in leaves of poplar, *Eucalyptus, etc.* Most favorable for their development was a stagnant atmosphere with high humidity and somewhat lowered oxygen content; only occasionally was cell enlargement combined with cell division. Injection of extracts from intumescence-forming tissue, of indoleacetic acid, *etc.*, also induced intumescence formation. A comparison with cell proliferation from wounded surfaces in various stem and leaf types led to the conclusion that in various types of hypertrophic growth (galls, intumescences, tyloses, lenticel proliferation, "Perldrüsen," *etc.*) auxin besides other factors may be the immediate cause of cell enlargement.

Cunningham (40), studying histological changes in wounded or infected leaves, found that in cases of fungus necrosis in leaf spot diseases a cicatrice was not always formed, even if the plant responded with periderm formation to mechanical injury of the leaves.

Saran (154) investigated the increase in respiration which occurs in leaves of *Anacardium occidentale* soon after wounding. Young

leaves (*e.g.*, 3, 8, 14 and 20 days old) respired normally at a higher rate than adult ones, but when wounded did not show an after effect. Older leaves (30, 40 and 100 days), on the other hand, showed a considerable increase in the respiratory rate after wounding, which passed off in about 2–4 hours. No histological details were given.

Audus (5) and Godwin (62) had previously shown that mechanical manipulation considerably increased the rate of respiration in cherry laurel leaves. A similar effect of mechanical stimulation was reported by Barker (9); slight mechanical pressure caused immediate increase in respiration of about 30% in shrivelled, soft-fleshed potatoes, and the rate did not return to normal for 10 days.

Hagemann (71) reviewed and investigated regeneration processes and location of root and shoot initials in a great number of leaf cuttings. The cause of the regenerative activities, according to the author, is to be sought rather in altered correlations due to isolation than in the wound stimulus or accumulation of food.

Schwarz (161) studied the structural changes in leaf cuttings, paying some attention to the problem of "directed" divisions. In petioles of *Coleus*, the plane of the wound surface or a center of necrosis does not determine the plane of divisions of cells situated between two vascular bundles, which is a continuation of the division plane of the fascicular cambium.

Fruits

Comparatively few reports on histological changes in wounded fruits are found in the older literature, although these fast and vigorously developing organs promise to be favorable material for such studies. A good deal of differentiation takes place during a relatively brief period, and great diversity of structures occurs. Of interest is the comparison of wound responses in the ovary, after fertilization, and in nearly mature fruits.

Küster (107) found long ago that the cells of the inner surface of the immature pericarp of Leguminosae (*e.g.*, *Pisum*, *Vicia*, *Lupinus*, *Lathyrus*, *Cytisus*) are very reactive. If the pods are opened and the inner parenchyma is exposed under moist conditions, the cells proliferate abundantly. In young pods masses of tissue develop and numerous cell divisions occur when a connection is made with the outside air by pricking.

Garms (61) investigated a great variety of fruit types (dry and

fleshy, dehiscent and non-dehiscent, types with stony or parenchymatous endocarp), studying mainly the healing processes in the fruit wall. Wound cork or callus were readily formed in both ovules and fruits. The most vigorous response generally took place when injury occurred after fertilization, that is, after the beginning of vigorous growth. Dicotyledons formed typical phellogen, while monocotyledonous fruits showed the modified type of storied cork. Meristematic activity was most vigorous near the moist interior of the fruits; the tissue formed here was in the form of callus, while periderm was formed in the more exposed outer regions. Some time before maturity was reached the cells lost the capability to divide, though a certain variability existed, depending on the fruit type. The elements of the epidermis were usually the first to lose the ability to divide.

Ulrich (178), in an extensive and valuable paper, reported similar results. Callus was formed best in fruits during rapid growth; too young fruits dropped after injury, too old ones decayed. In fruits of *Hedera helix* (179), confinement at high temperature, stagnant atmosphere, or the presence of certain gases in the air had a similar effect. Generally, presence of water vapor reduced cell division, suberization and lignification. The reactions were analogous to those known in leaves, stems, roots, *etc.*, in both physiological and histological respects. Abundant phelloderm was formed.

Spitzer (169) reported that the browning of the cut surface in apple and other fruits was due to the reaction of a chromogen and a specific enzyme.

Beth (12) investigated the effect of wounding on formation of adventitious embryos in ovaries and could not confirm Haberlandt's results in *Oenothera* where such embryos were formed after injury.

Nutman (137), however, studying changes in the developing embryo of rye, reported that the growth of the embryo sac is associated with a series of degenerative changes, and assumed that hormonal substances are liberated from the degenerative tissues. He suggested that lack of such substances may explain the arrested development of excised embryos of *Zizania aquatica* (115).

THE INDUCTION OF MERISTEMATIC ACTIVITY

Dedifferentiation

The following sections review briefly results of recent experi-

mental work concerning the various phases and phenomena of wound tissue formation as affected by different factors.

The return of mature cells to a more or less undifferentiated, meristematic condition occurs not infrequently during normal development. The process is usually accompanied by cell division and growth, and plays an important rôle in secondary meristematic activities, as in phellogen formation, anomalous thickening growth of stems, and in regeneration. Dissolution of endosperm membranes, of inner layers of gelatinous wood fibers, and of stone cells during the ripening of certain fruits (3) are other instances.

Dedifferentiation in these cases appears of a more or less normal and spontaneous nature, and in some of the instances mentioned the cells remain alive. The term, however, has also been applied to a different category of processes, also frequently met with in wound healing, when membranes and other remainders of dead cells become dissolved by the surrounding tissue. This occurs, for example, in calluses and grafts (105, 176, 59, 104), or when during vascular development in shoots and roots the first formed vessels become resorbed (35, 41). The breakdown of cell membranes may also be caused by typical necrobiotic processes (gummosis); sometimes this process is very slow (19).

That dedifferentiation may be readily induced in most mature plant cells by wounding is not only in itself a remarkable feature, but has stimulated a considerable amount of research in the hope that under such conditions the phenomena of cell rejuvenation, cell division and growth, may be analyzed and explained.

Striking examples of induced dedifferentiation in wound tissues are the cases in which specialized cells, often thick-walled or lignified, lose their impregnation and become thin-walled and embryonic, giving rise to daughter cells which often undergo a development distinctly different from their own.

Early statements that lignified cells can neither divide again (182) nor grow (156) can therefore hardly be maintained. Numerour reports by Trécul (177), Crüger (39), Schenk (157), Schilling (159) and others indicated that the contrary could often be observed in wound healing and related activities. In lignified vessels, sclerenchyma, and endodermal cells of various monocotyledons, wounding seems rather frequently to induce dedifferentiation (14, 16). It would thus appear that in higher plants somatic cells pre-

serve to a considerable degree developmental potencies which become realized when the cells are freed of their original correlations. The extent and the limitations of such dedifferentiation in plant cells are of great theoretical interest, although hardly systematically investigated.

Necrotic Changes and Wound Hormones

The traumatic stimulus produces, either directly or indirectly, a chain of rather heterogenous phenomena, at the beginning of which stand a number of conspicuous tissue changes near the wound surface, indicating the resumption or the stimulation of metabolic activity, but also some processes of a definite necrobiotic character. Cell expansion and cell division follow, though not necessarily together, and finally the complex of embryonic tissue once again differentiates in various ways, undergoing a process of physiological readaptation and chemical changes, of which structural redifferentiation is one visible expression. It appears obvious that interruption of tissue continuity by mechanical injury must set into motion numerous abnormal physical and chemical processes in the cells. As long as these are not sufficiently known any speculation regarding the true nature of the traumatic stimulus seems premature.

The various phases of necrotic and necrobiotic changes in the cells abutting on the wound surface are, however, of particular interest, for these processes play probably an important, though perhaps indirect, rôle in the induction of dedifferentiation and meristematic activity in the adjacent tissue. A variety of physical and physicochemical changes have been described here as early effects of injury, for example, changes in electrical potentials (106), vacuole contraction, increase in permeability and viscosity, and mechanical coagulation of the protoplasm (33, 109, 94, 104, 116, 162).

The outermost cells of the exposed tissue often atrophy and dry up quickly, but the cells and intercellular spaces beneath become infiltrated with necrobiotic products from the degenerating protoplasts, with tannin, suberin, enzymes and other substances; these products may become transported by means of vascular ducts to considerable distance into the healthy tissue, where secondary centers of meristematic activity are formed. The infiltration of air spaces is common, though Woodhead (194) takes exception to this observation. Decomposition products, wound gum and phlobaphenes,

also alter permeability and the chemical character of the membranes; oxygen and water have an effect on these necrobiotic changes (19).

Externally the necrotic zone shows a brownish or brownish-red discoloration; microchemically often a positive reaction is given with phloroglucin and hydrochloric acid, owing to the presence of varying amounts of certain aldehydes, whose relationship to lignin, however, is not definite. On the other hand, tannin and phlobaphenes often mask an original lignin reaction, and such cases have been erroneously described as delignification. These processes were investigated, for instance, in air roots of *Phoenix,* leaves of *Araucaria imbricata* (19), and in *Cereus* (160). Molisch described the formation of a red pigment from a chromogen in wound surfaces of Cactaceae (132).

Similar condensation and oxidation processes probably take place in the heartwood of trees; here Goodwin and Goddard (64) reported a low basal rate of oxygen consumption.

Dufrénoy (42) studied the relation of cellular immunity to the presence of phenolic compounds formed in wound tissues; he found that in highly resistant varieties, especially of potato, fungus penetration was checked, owing to the rapid death of cells and accumulation of phenolic compounds in adjacent ones which proved highly toxic to the parasite (compare also 32).

Watkins and Watkins (183) described lesions in cotton roots produced with unheated or heated extracts from *Phymatotrichum omnivorum.* In the case of unheated tissue extract an enzyme seems to be responsible for the breakdown of the walls. The effect was somewhat checked by the endodermis, but meristematic activity was induced in the pericycle.

These pathological changes seem to have important bearing on the problem of wound hormones. Research in this field has been very active, particularly experimental work based on the presence, or on the results of extraction and introduction of more or less specific, degenerative or natural substances from tissues. For testing the effectiveness of plant extracts and other chemical compounds promoting cell division various tissue and organ types have been used. A considerable number of organic and inorganic compounds promoting meristematic activity were reported, and ~~our~~ knowledge of natural growth substances has been greatly enriched by the results of extensive research on tissue and organ cultures (188, 47).

Early observations on cell divisions in the neighborhood of dying or necrotic cells indicated a causal connection, probably a chemical influence (189, 125). Haberlandt and his students demonstrated such effects in a series of well known experiments with isolated cells and tissue pieces, in which the presence of nutrients alone was not sufficient to induce cell division. Haberlandt termed these unknown substances wound hormones, although he was careful to emphasize the complexity of the traumatic stimulus at various occasions (68). He also suggested that wound substances were active in normal periderm formation (70) and related processes. A similar influence on cell division seemed to be exerted by the leptom (leptohormones). Fitting also has repeatedly emphasized the probability of a "hormonal" effect on protoplasmic streaming and respiration in wounded cells (*e.g.*, 50, 51, 52, 53).

Injection methods were successfully employed by Reiche (145) and have been perfected since (compare 150). Reiche injected tissue sap and cell debris into plants, which induced cell division and growth wherever necrotic deposits were formed within the healthy tissue. Numerous attempts have since been made to determine the nature of the effective agents. Wehnelt (184) used the parenchymatous (unwounded) layer of cells which line the seed chamber of the immature *Phaseolus* pericarp, to test the effectiveness of various substances in inducing cell division and proliferation. A variety of organic and inorganic compounds, including filtered or heated tissue sap from *Phaseolus* leaves, chicken albumen, horse serum, haemoglobin, insulin, agar, neutral salts, *etc.*, gave positive results.

Petri (142) repeated Haberlandt's experiments with *Echeveria* and potato and concluded that the wound hormones might be oxidation products of substances normally present in the cells.

Wilhelm (190) injected various substances into the parenchymatous pith cavity of *Vicia faba*, studying the formation of chromogen in the callus tissue thus induced. Besides tissue sap, various substances of heterogeneous chemical nature, such as sugar solution or horse serum, were found to be effective; the author concluded that the cells were unspecifically irritated, and a division hormone was secondarily produced within the plant.

Mrkos (133) studied the effect of agar containing a filtrate of *Rhizopus* cultures on bud regeneration in *Taraxacum* roots and on the depth at which periderm was formed under wound surfaces of *Bryophyllum* leaves.

Orth (140) confirmed Wilhelm's results; effective were tissue sap of *Vicia faba* and *Begonia,* also technical progynon and auxin.

Jost (90), mainly using *Phaseolus* material and the method of Wehnelt, showed that several organic acids, salts and indoleacetic acid also promote cell division and proliferation.

Weissenböck and Stern (185) studied the effect of indoleacetic acid injections on cell enlargement in pith and cortical cells of the *Phaseolus* epicotyl.

Orsós (139) found that higher breakdown products of proteins, such as heat-coagulated Kohlrabi protein, Witte pepton, fibrin, and also tyrosin induced cell divisions in sterile cultures of kohlrabi tissue; this result seemed to indicate that similar compounds, probably tyrosin, may be the active component in the wound hormone and leptohormone.

Several attempts were made to extract, concentrate and purify active substances which were assumed to be the more or less specific activators of cell division. Umrath and Soltys (180) isolated a substance from Papilionaceae which induced cell division in the bean pericarp, probably in low concentration (1: 50,000). This substance was described as an oxyacid with high oxygen content; it clearly differs from the active substances prepared from fresh beans by Bonner, English and Haagen-Smit (22, 44, 23, 45, 46). The first of these substances, termed traumatin, was extracted and purified, and had the chemical composition $C_{11}H_{17}O_4N$; it was heat-stable, and induced cell proliferation in the bean pod in as low a concentration as 1: 100,000. In a later paper (45) the isolation of an active dibasic acid of the composition $C_{12}H_{20}O_4$, a crystalline substance, was reported. Synthetic "traumatic acid" was identical in physiological activity with the natural product (46). The authors indicated that this substance also induced periderm formation in washed discs of the potato tuber. In their test, which was quantitative, the authors used the height of the intumescence, and thus cell growth, rather than the number of cell divisions as a measure of the activity of the substances. The positive reactions obtained with other substances, such as those used by Jost, Wehnelt and others were explained as due to non-specific injurious effects on the cells, which liberated wound hormones in them; in such cases, however, the callus was less high than after treatment with traumatin.

Hammett and Chapman (72) emphasized that the true characteristics of a wound hormone are the ability to stimulate cell division and the fact of liberation by the trauma itself rather than by disintegration. The fact that a compound promotes only tissue growth is not considered to be sufficient proof that it is a wound hormone. The sulfhydryl group fulfills the requirements of a wound hormone best. The authors found that in root tips of beans there was a correlation between increase in cell number (here termed proliferation) and SH concentration, while there was a negative correlation between SH concentration and root growth by cell elongation. The SH reaction was intensified by wounding.

Various workers expressed the opinion that the growth substances of the bios-complex are the ones most likely to fall into the category of wound hormones affecting cell division (*e.g.*, 186). Perhaps these were the substances missing in Haberlandt's early experiments in which cells only grew but did not divide. Rippel (147, 148, 149) studied the occurrence of bios in various seeds and seedlings, and suggested that they were probably of importance in callus formation, though he made tests only with yeast cells. Laird and West (110) found that a drop of bios 2(*b*) placed on the parenchymatous lining of the bean pod induced vigorous cell divisions.

Loofbourow and collaborators (121) reported the production of "proliferation-promoting factors" ("intercellular wound hormones") which were released by yeast cells after mechanical injury. As in their former studies, when yeast cells were subjected to lethal ultraviolet or X-rays, to heteroauxin or CO_2, the substances stimulated growth, fermentation and respiration of yeast; they were probably not disintegration products of dead cells, since they were produced while most of the cells were still living.

Ruhland and Ramshorn (153) studied the stimulating effect of expressed tissue juice on wound respiration. Application of juice to wound surfaces (leaves of *Begonia*, hypocotyls of *Helianthus*) increased the CO_2 production 5–10 times and the O_2 consumption 2–5 times. Some bios fractions from yeast had similar effects. The authors suggest that such substances might become postmortally active in wounded cells, and that wound hormones might thus be instrumental in regulating wound respiration.

To summarize, it would appear that the results reported in the foregoing section are still contradictory and that the intracellular

changes under the influence of the various substances, some of which might be wound hormones, are far from understood. Not only was the evidence obtained with very diverse plant material, but also often under unusual and unequal conditions. The immature bean pod tissue, which was used in the majority of cases, is a highly sensitive tissue of almost meristematic character; normally it is not exposed as in the experiments reported; if so, however, the cells, as in other Papilionaceae (107), readily proliferate and also multiply under changed relations of oxygen, water and probably temperature. It appears that some of the substances used in these tests have toxic effects, and easily destroy the cells; consequently little necrobiotic or meristematic activity follows. With substances assumed to be wound hormones the reaction was vigorous, but how these processes compare with those in tissue material in which after wounding extensive necrobiotic changes precede the occurrence of numerous cell divisions is open to question. Our knowledge of the physical and physico-chemical transmutations in injured and degenerating cells is still quite inadequate, and little is known about the character of natural substances and break-down products released into the adjoining healthy cells and how these may affect the complex mechanism of their metabolism and growth.

Cell Division

Cytokinesis. Some of the earliest visible effects of wounding are "traumatotactic" movements of nuclei and plastids toward the side of the cells facing the wound; furthermore, a considerable increase in the rate of cytoplasmic streaming. These processes are most conspicuous near the wound; after some time, though not always, redistribution of cytoplasm and nuclei may be noted (compare 57).

Nuclear changes which indicate the beginning of meristematic activity were studied by Birkholz (13), and especially by Heitz (76). This author expressed the opinion that the increase in nuclear growth is due to accumulation of sugar, and probably not to the action of wound substances.

Probably the increase in nuclear material goes hand in hand with that of the cytoplasm.

Fischer (48) reported that in leaves of *Peperomia* and *Bryophyllum* nuclear growth near the wound edge was due to swelling of the nucleus and increase in chromatic material. By supplying sugar the

enlargement of the nucleus and the beginning of mitotic division could be promoted.

The mechanism of cell wall formation and nuclear division in the early stages of wound tissue activity corresponds with that often described for large, vacuolate cells by Treub, Strasburger, Bailey and others (compare 165, 37). In such cells in which the nucleus is small compared with the cell volume, the phragmoplast and the cell plate are extended across the lumen of the mother cell by means of kinoplasmic fibrils at the periphery of the phragmoplast, until contact is made with the lateral wall. In polar view the system appears as a "halo," and the fact that at telophase the daughter nuclei appear in one plane has often caused erroneous reports on multinucleate condition in wound tissues.

Less has been known about the behavior of the cytoplasm and its inclusions during division. Sinnott and Bloch (166) studied the polarization of the cell and cytoplasm by the wound in large, vacuolate, dividing cells in traumatic tissues of *Tradescantia, Kalanchoë, Bryophyllum, Coleus, Phaseolus, Petunia,* and *Cucurbita,* in both living and fixed material. They found that the plane and position in which the cells are to divide is determined and visible in the cytoplasm from early prophase. Very soon after the migration of the rounded and enlarged nucleus into the center of the cell, plasma strands may be seen which aggregate and radiate from the nucleus outward toward the walls of the mother cell in the plane of the future division. Such a cytoplasmic diaphragm, the phragmosome, was found to persist during the successive stages of mitosis. Later the developing kinoplasmic fibrils and the cell plate follow exactly the course of the phragmosome until the lateral wall is reached. It was shown by the authors that this method of division is not limited to wound tissues, but occurs in meristems of many plant types, where comparatively large and vacuolate cells divide, *i.e.,* where the amount of cytoplasm is small in relation to the size of the cell (as in pith cells in growing stem tips).

Heitz (76) reported that in dividing and regenerating cells of bryophytes, nucleus and plastids are mechanically drawn to the center of the cells as a result of the redistribution of the cytoplasm. Before the nucleus entered prophase the chloroplasts assumed positions at the two nuclear poles and multiplied. He made similar observations in traumatic tissues of *Peperomia* and *Bryophyllum.*

Plane of Cell Division. The early divisions of wound meristems are generally parallel to the surface of the wound, *i.e.*, they occur at an angle of approximately 90 degrees to the direction of possible diffusion or other gradients from the wound surface. It may be assumed, therefore, that in this phase of cell divisions the plane is determined by the shape and influence of the wound itself, in both the ordinary wound phellogen and the wound cork of the storied type. Another interesting phenomenon in this early phase is the fact that the new walls are very often laid down almost exactly opposite each other in adjacent cells. In this manner the new division walls form concentric sheaths through all the living cells which are capable of rejuvenation, thus separating the necrotic part of the tissue from the healthy portion; this arrangement is found not only beneath external surface wounds, but also around inner centers of necrosis, *e.g.*, around infiltrated air spaces, vascular ducts, bast fibers and other elements.

Besides the wound stimulus there are other factors which influence plane and location of cell division. (I) In the neighborhood of vascular bundles which have been cut, or which run parallel and near to the wound surface, as well as in other more or less meristematic cell layers, such as pericycle and cambium, the cells divide more actively and nearer to the wound face than in other parts (*e.g.*, 138, 69, 7). This led to the assumption of active substances, *e.g.*, leptohormones diffusing from the bundles, but other factors, such as water and food relations, have here also been suggested. (II) In later phases of wound tissue activity the rules of parallelity and opposite position are often definitely abandoned, and cell division may occur in various planes, often coinciding with formation of root and shoot primordia. Here the plane of division is often related to the shape and direction of growth of the new cells. Cells released from pressure beneath the surface of a wound expand only in a radial and vertical direction and divisions occur only at an angle of 90 degrees to the longitudinal axis of the cell (96). Badian (7) emphasized that considerably later, when the original effect of the found stimulus is no longer exerted, divisions also occur in other directions, in cells whose long axis is not necessarily vertical to the wound surface.

Still another type of directed divisions during the later phases of wound tissue development coincides with the formation of tracheid

and phloem bridges. In wounded roots of *Taraxacum* (176), tracheids are thus formed from entire cortex cells or their segments. According to Simon (164), not only tracheids already present, but also meristems later to form tracheids have a directing and apparently stimulating influence on the formation of such tracheid connections. Kaan Albest (93) studied the direction of connecting strands of phloem and xylem in wounded stems of *Coleus, Impatiens, etc.* She concluded that the stimulation is probably exerted by sieve tubes and xylem vessels, respectively.

Cell Growth

The basic interrelationship between cell multiplication and cell growth typical for normal meristematic activity in plants may be considerably modified by external conditions during the various phases of wound tissue formation. Wound cork which shows comparatively little cell expansion may be considered to be in many surface tissues the usual form of activity after injury. Wound callus, on the other hand, is characterized by abundant growth and proliferation and comparatively little wall differentiation. It is often temporarily formed when under excessively humid conditions transpiration becomes reduced to a minimum, but temperature seems also to play a part. This type also occurs in deeply enclosed portions of the wound, or where the cut surfaces of an injury have remained in close contact.

The outer layers of the cork meristem often expand radially toward the surface, while the innermost cells may only divide. It does not appear impossible that the infiltration of intercellular spaces with sap somehow influences the growth activities of the cells in this area, in a manner perhaps analogous to the well known effect in growing tips of seedling roots where after irritation the intercellular spaces become infiltrated with liquid, and a marked inhibition of longitudinal growth follows and cell expansion takes place in the radial direction.

Between the various forms of cell proliferation, *e.g.*, callus, natural or induced tyloses, intumescences and other hyperhydric tissues, there is no sharp anatomical distinction. In their formation various factors have been found to be important, including availability of space, high water content of the air, temperature, variations in the oxygen and water relations (172), and chemical stimulation (107, 158, 167).

LaRue (111, 112, 113) investigated the conditions under which intumescences form, particularly the moisture relations, the influence of metabolic factors and the rôle of auxin. He gives a list of plants whose stems or leaves form intumescences (114) and found that the proliferating cell walls do not become thinned out and therefore actively grow.

A simple form of "inner intumescences" often discussed in the older literature are tyloses (compare 120). They are very common, and Meyen (128) included under this term not only the natural tyloses, which, for example, occlude the vessels in ageing organs, but all other cells which proliferate into any cavity. These are usually formed after experimental wounding, where they may be seen growing into vessels, obliterating air passages, necrotic parenchyma cells, *etc.* (39, 21, 67, 81, 26). They are often combined with cell divisions and show a close relationship to the formation of necrotic and decomposition products and "wound gum" (131, 14, 17, 19, 20, 27, 1, 2). Sometimes the anatomical evidence suggests that the growth of tyloses is proceeding under some chemotropical stimulus, for instance, in material figured by Reiche (145), where tylosis-shaped cells grow along and toward surfaces covered with cell debris and decomposition products. The manner of wall growth in tyloses is somewhat comparable to the type of "intrusive growth" of cells often observed in normal tissue readjustments (*e.g.*, 135, 165).

Cell and Tissue Differentiation

The effects of wounding on differentiation are many-fold and vary considerably with the age of the organ and the distance from the injury. In young organs differentiation taking place near the injury at the time of wounding may become arrested or diverted (*e.g.*, 55, 56, 193), while an accelerating effect of the wound on metabolic activity and on structural differentiation is almost invariably seen in layers further distant from the wound beyond the zone of cells made to divide more or less actively. In older organs the effects of wounding are naturally somewhat modified and, as mentioned before, increased differentiation may be the only wound response.

The differentiation processes in the wound meristem proper take many forms. If wound periderm is formed, differences specific for

the plant type occur with regard to the sequence and manner of cell division (*e.g.*, unequal divisions; storied cork; phellogen). Cell division is followed by various types of wall differentiation, especially in plants whose normal cork has these qualities.

In extensive tissue regeneration redifferentiation may be very complex. Thus Vöchting (181) has shown that the pith of the kohlrabi tuber is capable of regenerating completely the primary tissue arrangement of the tuber with all its specific elements; even a new epidermis with stomata becomes differentiated beneath the first formed phellogen.

Finally a considerable number of cases have become known in which structural redifferentiation and restoration of anatomical features is less complicated. These cases have in common the fact that in the neighborhood of the wound the cells resume development under the accelerating influence of the wound stimulus, and they later age like normal surface tissues by thickening of the membranes and by impregnation with suberin and lignin, usually in a manner characteristic for the particular plant. This has the striking result that to a greater or lesser extent the primary tissue pattern becomes restored. In some plant types all layers of the tissue do not react in the same manner; certain layers of the meristem or of the ground tissue actually exhibit a specific reactivity in this process.

Noteworthy examples of such behavior are: petioles and stems of ferns (80, 82, 83); leaves of orchids (25, 193); air roots and stems of monocotyledons (14, 16, 17, 19); fruits (61, 178); cork of Cactaceae (130, 136, 38, 77); roots of *Chelone* (123); stem of *Kleinia* (194); submerged monocotyledons (151). In wound callus and phelloderm of fruits the differentiation processes are still quite varied (61): wall thickening and impregnation, formation of specific forms of sclereids, crystal cells, tracheids, secretory ducts. With increasing age here the wound periderm becomes histologically and microchemically more and more similar to normal surface tissue; its contents, such as chlorophyll, starch, tannin, crystals, anthocyanin and fat, undergo changes similar to those in unwounded parts.

In air roots of *Philodendron Glaziovii* (14) the wound meristem derives from older cortical cells, and in their division products the redifferentiation of pitted, thick-walled, subepidermal sclereids occurs, which link up with the original sclerenchyma sheath present at the wound edge. These cells may make their appearance in the

wound meristem of young roots at a time at which the original sheath at the other sides of the root has not yet appeared. Similarly in wounded air roots of *Phoenix reclinata* (19) cells within the wound tissue develop U-shaped thickenings, and these cells link up with the original superficial sclerenchyma. In the wound surfaces microchemical changes also are similar to those normally taking place. Similar behavior has been observed in roots of *Iris*, and stems and roots of *Tradescantia fluminensis*. Air roots of orchids do not form wound cork at all, but if the wounds penetrate through the exodermis as far as the living cortex, the already mature cortex cells thicken and lignify in the same manner as normal exodermis cells (16). In the instances just described cells originally destined for a different function seem to have undergone a true process of redifferentiation. There seems little doubt that the factors which bring about such structural redifferentiation must be related to those which are instrumental in normal development. The exact nature and control of this complicated mechanism are not known. In wound tissues the restoration of anatomical features near the surface appears to be linked up with oxygen and water relations which determine the fate of the dedifferentiated meristematic cells. Under favorable conditions there seems to exist some similarity with normally developing organs, and condensation and oxidation processes within the cells result in microchemical changes making them very similar to normal structures.

In layers such as bundle sheaths, pericycle, endodermis, *etc.*, differentiation is particularly likely to become accelerated by wounding. Thus in *Tradescantia fluminensis* the internal bundle sheaths which are normally thin-walled sclerify after wounding (17); this character is normally present in other members of the Commelinaceae. Other examples of characters not normally apparent but induced by wounding are the traumatic resin ducts of certain Abietineae (*Tsuga*, *Sequoia*). These are regarded by Jeffrey (86) and his school (see also 8) as reversions to an ancestral condition normally present in other members of the family.

GROWTH SUBSTANCES AND WOUNDING

Recent reports on work with natural and synthetic growth substances have often referred to histological changes comparable to wound structures. Some results obtained with growth substances

(*e.g.*, auxin and indoleacetic acid) in inducing cell division and growth in parenchymatous tissue of *Phaseolus* and *Vicia* have been considered under the heading of wound hormones. But high concentrations of growth substances or growth substance mixtures (79) have been very generally applied in lanolin to decapitated and other wound surfaces, in which case vigorous reactions resulted due to the combined action of tissue interruption and chemical irritation. In a number of cases the effects of the growth substances were noticed at a considerable distance from the point of application, but the formation of the wound meristem did not seem to be influenced. In other instances natural meristematic activities, such as the formation of abscission layers, became even inhibited (60). In the majority of cases, however, the changes which were due to the treatment by the chemicals overshadowed the original wound reactions in the region of the injury, inducing especially vigorous cell division and growth in those cells which were normally very active in processes of wound healing and regeneration. It appears, therefore, that the stimulus provided by the growth substances may be substituted for other stimuli which exert comparable effects. The qualitative character of the histological changes is thus determined by the inherent formative organization of the plant rather than by specific properties of the chemical agents. Several workers have emphasized, from similar considerations, the non-specificity and the irritant character of many growth substances (90, 54, 6, 20, 78, 173).

Studies of histological changes similar to wound responses following growth substance treatment of wounds are contained in the following papers. Monocotyledons: *Lilium* (10, 11), *Tradescantia fluminensis* (20); Dicotyledons: *Iresine* (75), *Phaseolus* and tomato (118), tomato (24), *Phaseolus* (103), *Brassica* (84, 63).

The formation of vigorous callus growth of crown gall-like character was induced by high concentrations of growth substances in *Phaseolus* (102, 30, 31, 74) and in *Mirabilis* (73).

According to Jakeš and Hexnerová (85), callusing of ringing wounds in fruit trees can be improved by applying 1% indoleacetic acid or "Belvitan" paste to the surface of the exposed wood.

The rôle of polarity in wound callus, root and shoot formation of woody cuttings treated with growth substances was investigated by Rogenhofer (152), Fischnich (49) and Zimmerman and Hitchcock (200).

Brown and Carmack (29) also studied cambial activity in relation to a wound and to indoleacetic acid treatment.

Jost (91) reported on vessel development in decapitated epicotyls of *Phaseolus* treated with indoleacetic acid, and found that growth substances stimulated cambial activity only in general, the differentiation into vessels depending on other factors.

PLANT NEOPLASMS

The "tumorous" overgrowths obtained by prolonged or repeated treatment with growth substances often form a histological transition to related types of anomalous neoplastic growths induced by various chemical irritants or organisms, such as *Bacterium tumefaciens* (*e.g.*, 117). Besides certain systemic responses, this organism causes the formation of an unorganized type of gall; such pathological forms have often been compared with malignant neoplastic growths in animals. In the etiology of plant neoplasms, however, the significant factor is the continued presence of the irritant, the growth coming to an end when the causing agent ceases to be active; the analogy with an inflammatory tissue condition in animals is obvious. The early stages show striking resemblance to wound reactions (146); under prolonged irritation callus masses of unusual dimensions develop which, however, age and differentiate in various ways, often producing root and shoot primordia. The qualitative character of such forms also appears determined by the type of injury and the mode of growth of the particular plant type rather than by a specific action of the irritant or the inciting organism (95, 117).

Also the spontaneous, hereditary tumors of *Nicotiana* hybrids have close histological relationship to chemically induced overgrowths and to crown-gall, and often arise on wounds, whatever the genetic base of the malformations may be (98, 101).

The organizing and reconstructing tendency in plant tissues, which is characteristic of wound healing and plays a conspicuous rôle in anomalous overgrowths, finally finds still other methods of expression in the products of certain gall-inducing insects (compare 34). Here often normal cell forms and tissue arrangement become specifically modified and controlled to a remarkable extent under the influence of the inciting organism, but our knowledge as regards the interaction in this process of host and parasite is still very meager.

The occurrence of polyploid cells in tumors of hereditary, parasitic or chemical origin has been frequently reported. Comparatively few studies were made of related changes in wound and callus tissue.

Winkler's (192) method of obtaining polyploid shoots from somatic cells by wounding was successfully developed by Jørgensen (89) and has been employed since in horticultural practice.

Kostoff (97) suggested that polyploidy and other irregularities in cell division were due to factors which increase the cytoplasmic viscosity in the callus tissue.

Grafl (66) recently investigated ploidy in the tuber of *Sauromatum guttatum*. She found large 16-ploid nuclei beneath wound surfaces, and noted also dividing large nuclei which were tetra- or octoploid in the normal tissue. These facts suggest that multiplication of chromosomes during the resting stage is probably a general phenomenon of tissue differentiation in higher plants, and this may be demonstrated by wounding.

It is possible that in such cases the nuclear changes may be the result of progressive cell enlargement rather than the cause of it, and similar conditions might apply to cases of polyploidy observed in hypertrophic cells after treatment with indoleacetic acid, also in some galls and root nodules. Such nuclear changes would thus fall into a category somewhat different from the types of atypical growth in which chromosomal aberrations have been suggested as the primary cause of histological anomalies (*e.g.*, 99, 100, 88).

SUMMARY

The research reviewed in the foregoing paper deals with theoretical and practical aspects of wound healing in higher plants, *i.e.*, with the tissue changes which take place adjacent to cells and tissues damaged by various artificial and natural accidents.

Recent work has been particularly concerned with experimentally induced wound repair in different plant types, in so far as such studies are able to throw light on the problem of cellular reactivity and the physiological basis of plant structure.

The study of dedifferentiation, meristem formation and redifferentiation in vacuolate cells has also proved to be a helpful tool in the elucidation of the mechanics of cell division and differentiation.

The first part of this paper reviews more or less related, practical

and theoretical studies on wound healing in plants of different structure and reactivity under different conditions. The results in stems, root leaves, fruits, *etc.*, show that metabolic activities and the quality of the protective tissues formed are correlated with the mode of growth and the manner of distribution of meristematic and potentially meristematic zones and cell layers in the plant.

The age of the plant and seasonal variations affect wound healing. More especially the external conditions (light, temperature, gravity, access of oxygen and humidity), furthermore the internal environment (the presence of leptom elements and vascular ducts, water supply, food distribution, pH, enzymes and growth substances) influence the wound reaction both qualitatively and quantitatively.

In the second part of the paper the phenomena of meristematic activity, dedifferentiation, cell division and growth, and cell and tissue redifferentiation are considered in more detail. Reference is made to the morphogenetic problems involved in these various phases of wound tissue formation.

Contemporary research has shown that dedifferentiation may be induced in most mature, living cells by wounding. Even specialized cells, such as thick-walled lignified elements may in this process become delignified and thin-walled. Another type of pathological dedifferentiation is the postmortal breakdown of cells and cell walls under the influence of enzymes from necrobiotic cells.

In spite of the progress made in the analysis of changes in cells abutting on the wound surface, the exact nature of the traumatic stimulus and of the physical and physico-chemical changes in wounded cells is still unknown. Of interest are the necrotic and necrobiotic processes in the surface layers of the wound, such as the infiltration of intercellular spaces with sap and decomposition products, which in their turn seem to affect respiration, cell growth and division in adjacent healthy cells. Water and oxygen seem to play a limiting rôle in these processes.

Recent research on wound hormones has shown that numerous organic and inorganic compounds, some of them occurring naturally in plants, are capable of influencing metabolic processes and inducing meristematic activity, if applied to the exposed surface of the seed cavity of the *Phaseolus* pericarp or to other tissues. Certain heat-stable preparations from tissue extracts were particularly effective.

Cell division is always parallel to the surface of the wound or internal centers of necrosis in the early phase of wound tissue activity. It was shown that the polarizing influence from the wound is exerted upon the cell as a whole and that the plane of division becomes visible by the configuration of the cytoplasm before it becomes evident in the mitotic figure.

Cell growth becomes more prominent in humid and stagnant atmosphere; it is also more pronounced in the outer zone of the wound meristem, whose intercellular spaces have become injected with liquid after wounding, and it is typical for internal meristematic activity, for example, tyloses.

In developing young organs differentiation may become inhibited in cells abutting on the injury, but it is usually promoted in cells further distant.

The cells of the wound meristem often redifferentiate in a manner characteristic for the particular plant, but the derivatives of dedifferentiated cells may redifferentiate in a fashion distinctly different from the parent cell. In older organs and certain plant types, differentiation, *e.g.,* thickening and impregnation of the wall, may be the only wound response. The process of differentiation and of restoration of anatomical features near the wound surface appears often to be largely a result of metabolic and microchemical changes, and of oxidation and condensation processes in the cells similar to those occurring under normal differentiation of the organ.

A part of the recent literature on growth substances has been concerned with anomalous tissue structures induced by the application of growth substances to wounds. These, as well as other plant tumors of chemical and bacterial origin, are discussed and reference is made to their developmental and histological relationship to wound structures. Recent reports on polyploidy in wound tissues are reviewed.

* * * *

DEFINITIONS

Differentiation: Appearance of differences in the physiological and morphological character of cells.

Dedifferentiation: Process by which mature or specialized cells lose their differentiated character and rejuvenate.

Redifferentiation: Physiological and structural differentiation of dedifferentiated cells.

Necrosis: Rapid, atrophic death of cells.

Necrobiosis: Gradual, degenerative death of cells, characterized by abnormal metabolic changes and postmortal activity of enzymes.

Phragmosome: Cytoplasmic diaphragm, formed early during division of vacuolate cells in the position of the future division wall.

BIBLIOGRAPHY

1. ALEXANDROV, W. G., AND ALEXANDROVA, O. G. Ist die Verholzung ein reversibler oder irreversibler Vorgang? Planta **7**: 340–346. 1928.
2. ———, AND ———. Über Thyllenbildung und Obliteration bei Spiralgefässen. Beitr. Biol. Pflanz. **17**: 393–403. 1929.
3. ———, AND DJAPARIDZE, L. I. Über das Entholzen und Verholzen der Zellhaut. Planta **4**: 467–475. 1927.
4. ARTSCHWAGER, ERNST. Wound periderm formation in the potato as affected by temperature and humidity. Jour. Agr. Res. **35**: 995–1000. 1927.
5. AUDUS, L. J. Mechanical stimulation and respiration rate in the cherry laurel. New Phytol. **34**: 386–402. 1935.
6. AVERY, G. S., JR. La notion des phytohormones. Leurs rapports avec les stimulants et l'irritabilité des plantes. Études et recherches sur les phytohormones. Inst. Int. Coop. Intell. Soc. Nations. 49–54. 1938.
7. BADIAN, J. Ueber Zellteilungen in verwundeten Keimblättern. Acta Soc. Bot. Pol. **14**: 87–115. 1937.
8. BANNAN, M. W. Seasonal wounding and resin-cyst production in the hemlock, *Tsuga canadensis* (L.) Carr. Ann. Bot. **48**: 857–868. 1934.
9. BARKER, J. A note on the effect of handling on the respiration of potatoes. New Phytol. **34**: 407–408. 1935.
10. BEAL, J. M. Bud development in *Lilium harrisii* following treatment with indoleacetic acid. Proc. Nat. Acad. Sci. **23**: 304–306. 1937.
11. ———. Histological responses of three species of *Lilium* to indoleacetic acid. Bot. Gaz. **99**: 881–911. 1938.
12. BETH, K. Untersuchungen über die Auslösung von Adventivembryonie durch Wundreiz. Planta **28**: 296–343. 1938.
13. BIRKHOLZ, E. Wundreiz und Kernveränderung. Protoplasma **13**: 282–321. 1931.
14. BLOCH, R. Umdifferenzierungen an Wurzelgeweben nach Verwundung. Ber. Deut. Bot. Ges. **44**: 308–316. 1926.
15. ———. Zum Problem der Korkentstehung. Ber. Deut. Bot. Ges. **44**: 316–321. 1926.
16. ———. Observations on the relation of adventitious root formation to the structure of air-roots of orchids. Leeds Phil. Soc. Proc., Sci. Sec. Vol. 3, Part 2: 92–101. 1935.
17. ———. Wound healing in *Tradescantia fluminensis* Vell. Ann. Bot. **49**: 651–670. 1935.
18. ———. The natural wounds in the leaves of *Araucaria imbricata* Pavon. The Naturalist (North of England): 193–199. Sept. 1936.
19. ———. Wound healing and necrosis in air roots of *Phoenix reclinata* and leaves of *Araucaria imbricata*. Am. Jour. Bot. **24**: 279–287. 1937.
20. ———. Anatomical changes in *Tradescantia fluminensis* Vell. after treatment with growth substances. Boyce Thomp. Inst. Contr. **9**: 439–454. 1938.
21. BOEHM, J. Über Function und Genesis der Zellen in den Gefässen des Holzes. Sitz. Ber. K. AK. Wiss. Wien, Abt. II. **55**: 851–866. 1867.

22. Bonner, J. and English, J., Jr. Purification of traumatin, a plant wound hormone. Science **86**: 352–353. 1937.
23. ———, and ———. A chemical and physiological study of traumatin, a plant wound hormone. Plant Physiol. **13**: 331–348. 1938.
24. Borthwick, H. A., Hamner, K. C., and Parker, M. W. Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. Bot. Gaz. **98**: 491–519. 1937.
25. von Bretfeld. Über Vernarbung und Blattfall. Jahrb. Wiss. Bot. **12**: 133–160. 1880.
26. Brieger, A. Untersuchungen über den Wundreiz. I. Ber. Deut. Bot. Ges. **42**: (79)-(90). 1924. II. Die Ätiologie der Thyllen. Ber. Deut. Bot. Ges. **43**: 443–449. 1925.
27. Brockhuizen, S. Wondreacties van hout. Het ont staan van thyllen en wondgom, in het bizonder in verband met iepenziekte. Diss. Utrecht. 1929.
28. Brown, A. B. Activity of the vascular cambium in relation to wounding in the balsam poplar, *Populus balsamifera* L. Canad. Jour. Res. **15**: 7–31. 1937.
29. ———, and Carmack, R. G. H. Stimulation of cambial activity, locally in the region of application and at a distance in relation to a wound by means of heteroauxin. Canad. Jour. Res. **15**: 433–441. 1937.
30. Brown, N. A., and Gardner, F. E. Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. Phytopath. **26**: 708–713. 1936.
31. ———, and ———. Indoleacetic acid galls of a secondary type. Phytopath. **27**: 1110–1113. 1937.
32. Brown, W. The physiology of host-parasite relations. Bot. Rev. **2**: 236–281. 1936.
33. Bünning, E. Untersuchungen über die Koagulation des Protoplasmas bei Wundreizen. Bot. Arch. **14**: 138–164. 1926.
34. Carter, W. Injuries to plants caused by insect toxins. Bot. Rev. **5**: 273–326. 1939.
35. Chauveaud, G. L'appareil conducteur des plantes vasculaires et les phases principales de son evolution. Ann. Sci. Nat. Bot. IX. **13**: 113–436. 1911.
36. Collins, J. F. Treatment and care of tree wounds. U. S. Dept. Agr. Farmers Bull. 1726. 1934.
37. Conard, A. Sur un nouveau mode de formation de la membrane dans les tissus cicatriciels d'une feuille. Bull. Classe Sci. Acad. Roy. Belgique V. **8**: 531–541. 1922.
38. Coutant, M. W. Wound periderm in certain cacti. Bull. Torr. Bot. Club **45**: 353–364. 1918.
39. Crüger, H. Einiges über Gewebeveränderungen bei der Fortpflanzung durch Stecklinge. Bot. Zeit. **18**: 369–374. 1860.
40. Cunningham, H. S. Histology of the lesions produced by *Sphaceloma Fawcettii* Jenkins on leaves of Citrus, and A study of the histological changes in leaves by certain leaf-spotting fungi. Phytopath. **18**: 539–545; 717–751. 1928.
41. Dauphiné, A. Recherches sur les variations de la structure des rhizomes. Ann. Sci. Nat. Bot. IX. **3**: 317–368. 1906.
42. Dufrénoy, J. Cellular immunity. Amer. Jour. Bot. 23: 70–79. 1936.
43. Eglits, M. Der Einfluss der Infektion auf die Temperatur und die Kohlensäureabgabe bei Kartoffeln. Phytopath. Ztschr. **5**: 343–379. 1932.
44. English, J., and Bonner, J. The wound hormones of plants. I. Traumatin, the active principle of the bean test. Jour. Biol. Chem. **121**: 791–799. 1937.

45. ———, ———, AND HAAGEN-SMIT, A. J. The wound hormones of plants. II. The isolation of a crystalline active substance. Proc. Nat. Acad. Sci. **25**: 323–329. 1939.
46. ———, ———, AND ———. Structure and synthesis of a plant wound hormone. Science **90**: 329. 1939 (see also Jour. Am. Chem. Soc. **61**: 3434–3436. 1939).
47. FIEDLER, H. Die pflanzliche Gewebe- und Organkultur. Ztschr. Bot. **33**: 369–416. 1938.
48. FISCHER, H. Grössenveränderungen von Kern und Nucleolus im Blattgewebe. Planta **22**: 767–793. 1934.
49. FISCHNICH, O. Weitere Versuche über die Bedeutung des Wuchsstoffes für die Adventivspross- und Wurzelbildung. Ber. Deut. Bot. Ges. **57**: 122–134. 1939.
50. FITTING, H. Untersuchungen über die Auslösung von Protoplasmaströmung. Jahrb. Wiss. Bot. **64**: 281–388. 1925.
51. ———. Untersuchungen über den Protoplasmaströmung auslösenden Reizstoff der Pflanzen. Naturwiss. **21**: 489–495. 1933.
52. ———. Über Auslösung von Protoplasmaströmung bei *Vallisneria* durch einige Histidinverbingungen. Jahrb. Wiss. Bot. **82**: 613–624. 1936.
53. ———. Beiträge zur Physiologie der Protoplasmaströmung in den Blättern von *Vallisneria spiralis*. Ber. Deut. Bot. Ges. **55**: 255–261. 1937.
54. ———. Die Hormone als physiologische Reizstoffe. Biol. Zentralblatt **56**: 69–86. 1936.
55. FOURCROY, M. Action indirecte des traumatismes sur l'évolution de l'appareil conducteur. Compt. Rend. Acad. Sci. **196**: 210–212. 1933.
56. ———. Reconstitution de la structure normale dans des racines traumatisées. Rév. Gén. Bot. **45**: 159–170. 1933.
57. FRANK, B. Über die Veränderung der Lage der Chlorophyllkörner in der Zelle und deren innere und äussere Ursachen. Jahrb. Wiss. Bot. **8**: 216–303. 1872.
58. FRITZ, F. Über die Kutikula von *Aloë* und *Gasteria*arten. Jahrb. Wiss. Bot. **81**: 718–746. 1935.
59. FUNK, R. Untersuchungen über heteroplastische Transplantationen bei Solanaceen und Cactaceen. Beitr. Biol. Pfl. **17**: 404–468. 1929.
60. GARDNER, F. E., MARTH, P. C., AND BATJER, L. P. Spraying with plant growth substances to prevent apple fruit dropping. Science **90**: 208–209. 1939.
61. GARMS, H. Untersuchungen über Wundheilung an Früchten. Beih. Bot. Centralbl. I. **51**: 437–516. 1933.
62. GODWIN, H. The effect of handling on the respiration of cherry laurel leaves. New Phytol. **34**: 403–406. 1935.
63. GOLDBERG, E. Histological responses of cabbage plants grown at different levels of nitrogen nutrition to indole-3-acetic acid. Bot. Gaz. **100**: 347–369. 1938.
64. GOODWIN, R. H., AND GODDARD, D. R. The oxygen consumption of isolated woody tissues. Amer. Jour. Bot. **27**: 234–237. 1940.
65. GORE, U. R., AND TAUBENHAUS, J. J. Anatomy of normal and acid injured cotton plants. Bot. Gaz. **92**: 436–441. 1931.
66. GRAFL, I. Kernwachstum durch Chromosomenvermehrung als regelmässiger Vorgang bei der pflanzlichen Gewebedifferenzierung. Chromosoma **1**: 265–275. 1939.
67. GÜRTLER, F. Über interzellulare Haarbildungen. Diss. Berlin. 1905.
68. HABERLANDT, G. Culturversuche mit isolierten Pflanzenzellen. Sitz. Ber. K. Akad. Wiss. Wien. Math.-nat. Classe. 111. I: 69–91. 1902.
69. ———. Zur Physiologie der Zellteilung. Zweite Mitteilung. Sitz. Ber. K. Pr. Akad. Wiss. Phys.-math. Classe. **46**: 1096–1111. 1914.

70. ———. Zur Entwicklungsphysiologie des Periderms. Sitz. Ber. Pr. Akad. Wiss. Phys.-math. Klasse 317–338. 1928.
71. HAGEMANN, A. Untersuchungen an Blattstecklingen. Gartenbauwiss. **6**: 69–195. 1932.
72. HAMMETT, F. S. AND CHAPMAN, S. S. A correlation between sulfhydryl, mitosis, and cell growth in length in roots of *Phaseolus vulgaris*. Growth **2**: 297–302. 1938.
73. HAMNER, K. C. Histological responses of *Mirabilis jalapa* to indoleacetic acid. Bot. Gaz. **99**: 912–954. 1938.
74. ———, AND KRAUS, E. J. Histological reactions of bean plants to growth promoting substances. Bot. Gaz. **98**: 735–807. 1937.
75. HARRISON, B. F. Histological responses of *Iresine Lindenii* to indoleacetic acid. Bot. Gaz. **99**: 301–338. 1937.
76. HEITZ, E. Das Verhalten von Kern und Chloroplasten bei der Regeneration. Zeits. Zellf. **2**: 69–86. 1925.
77. HEMENWAY, A. F. An anatomical study of traumatic and other abnormal tissues in *Carnegiea gigantea*. Amer. Jour. Bot. **21**: 513–518. 1934.
78. HITCHCOCK, A. E. AND ZIMMERMAN, P. W. Unusual physiological responses induced on intact plants by capping with black cloth. Boyce Thompson Inst. Contr. **10**: 389–398. 1939.
79. ———, AND ———. Effects obtained with mixtures of root-inducing and other substances. Boyce Thompson Inst. Contr. **11**: 143–160. 1940.
80. HOLDEN, H. S. Some wound reactions in Filicinean petioles. Ann. Bot. **26**: 777–793. 1912.
81. ———. On the occurrence of cavity parenchyma and tyloses in ferns. Jour. Linn. Soc. Bot. **47**: 141–153. 1925.
82. ———. Observations on some wound reactions in the aerial stem of *Psilotum triquetrum*. Ann. Bot. **44**: 285–296. 1930.
83. ———. Some observations on the wound reactions of *Ankyropteris corrugata*. Jour. Linn. Soc. **48**: 643–655. 1931.
84. HOWARD, H. W. Possible action of phytohormones as root-determiners. Ann. Bot. **2**: 934–942. 1938.
85. JAKEŠ, E. AND HEXNEROVÁ, H. Über den Einfluss der Wuchsstoffe auf Wundgewebebildung bei Obstbäumen. Gartenbauwiss. **13**: 83–93. 1939.
86. JEFFREY, E. C. The comparative anatomy and phylogeny of the coniferales. Part I. The genus *Sequoia*. Mem. Boston Soc. Nat. Hist. **5**: 441–459. 1903.
87. JOHNSTONE, G. R. Effect of wounding on respiration and exchange of gases. Bot. Gaz. **79**: 339–340. 1925.
88. JONES, D. F. Nuclear changes affecting growth. Amer. Jour. Bot. **27**: 149–155. 1940.
89. JØRGENSEN, C. A. The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Genet. **19**: 132–210. 1928.
90. JOST, L. Wuchsstoff und Zellteilung. Ber. Deut. Bot. Ges. **53**: 733–750. 1935.
91. ———. Zur Physiologie der Gefässbildung. Zeits. Bot. **35**: 114–150. 1940.
92. JUMP, J. A. Wound responses of *Ficus australis*. Bull. Torr. Bot. Club **63**: 477–481. 1936.
93. KAAN, ALBEST A. VON. Anatomische und physiologische Untersuchungen über die Entstehung von Siebröhrenverbindungen. Zeits. Bot. **27**: 1–94. 1934.
94. KEIL, R. Über systolische und diastolische Veränderungen der Vakuole in der Zelle höherer Pflanzen. Protoplasma **10**: 568–597. 1930.
95. KISSER, J. Über die Wirkungen carcinogener Substanzen bei Pflanzen. Ber. Deut. Bot. Ges. **57**: 506–515. 1939.

96. Kny, L. Über den Einfluss von Zug und Druck auf die Richtung in sich theilenden Pflanzenzellen. Ber. Deut. Bot. Ges. **14**: 378–391. 1896.
97. Kostoff, D. Protoplasmic viscosity in plants. II. Cytoplasmic viscosity in callus tissue. Protoplasma **11**: 184–189. 1930.
98. ———. Tumor problem in the light of researches on plant tumors and galls and its relation to the problem of mutation. Protoplasma **20**: 440–456. 1933.
99. ———. Abnormal mitosis in tobacco plants forming hereditary tumors. Nature **144**: 599. 1939.
100. ———. Studies on atypical growth in plants from a cytogenetic point of view. Jour. Genet. **39**: 469–484. 1940.
101. ———, and Kendall, J. Studies on plant tumors and polyploidy produced by bacteria and other agents. Arch. Mikrobiol. **4**: 487–508. 1933.
102. Kraus, E. J., Brown, N. A., and Hamner, K. C. Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. **98**: 370–420. 1936.
103. ———, and Mitchell, J. W. Histological and physiological responses of bean plants to alpha naphthalene acetamide. Bot. Gaz. **101**: 204–225. 1939.
104. Krenke, N. P. Wundkompensation, Transplantation und Chimären bei Pflanzen. 1933.
105. Krieg, A. Beiträge zur Kenntnis der Kallus- und Wundholzbildung geringelter Zweige und deren histologische Veränderungen. Diss. Würzburg. 1908.
106. Kümmel, K. Elektrische Potentialdifferenzen an Pflanzen. Planta **9**: 564–630. 1930.
107. Küster, E. Histologische und experimentelle Untersuchungen über Intumeszenzen. Flora **96**: 526–537. 1906.
108. ———. Pathologische Pflanzenanatomie. 3. Aufl. 1925.
109. ———. Pathologie der Pflanzenzelle. I. Protopl. Monogr. 1929.
110. Laird, D. G., and West, P. M. The influence of bios on nodule bacteria and legumes. A. The influence of bios on legume seedlings. Canad. Jour. Res. **15**: 1–6. 1937.
111. LaRue, Carl D. Intumescences on poplar leaves. I. Structure and development. II. Physiological considerations. III. The rôle of plant hormones in their production. Amer. Jour. Bot. **20**: 1–17; 159–175. 1933. **23**: 520–524. 1936.
112. ———. Intumescences on leaves of *Eucalyptus cornuta, E. coccifera, Hieracium venosum, Mitchella repens,* and *Thurberia thespesioides.* Phytopath. **23**: 281–289. 1933.
113. ———. The part played by auxin in the formation of internal intumescences in the tunnels of leaf miners. Bul. Torr. Bot. Club **64**: 97–102. 1937.
114. ———. Cell outgrowths from wounded surfaces of plants in damp atmospheres. Papers Mich. Acad. Sci. **22**: 123–139. 1937.
115. ———, and Avery, G. S., Jr. The development of *Zizania aquatica* in seed and in artificial culture. Bull. Torr. Bot. Club **65**: 11–21. 1938.
116. Lepeschkin, W. W. Zell Nekrobiose und Protoplasmatod. Protopl. Monogr. 1937.
117. Levine, M. Plant responses to carcinogenic agents and growth substances; their relation to crown gall and cancer. Bull. Torr. Bot. Club **67**: 199–226. 1940.
118. Link, G. K. K., Wilcox, H. W., and Link, A. D. Responses of bean and tomato to *Phytomonas tumefaciens, P. tumefaciens* extracts, β-indoleacetic acid, and wounding. Bot. Gaz. **98**: 816–867. 1937.

119. LINSBAUER, K. Betrachtungen zum Problem der Sprossregeneration. Planta **9**: 334–338. 1929.
120. LOHSE, R. Entwurf einer Kritik der Thyllenfrage mit Ergebnissen eigener Versuche. Bot. Arch. **5**: 345–380. 1924.
121. LOOFBOUROW, J. R., COOK, E. S., DWYER, C. M., AND HART, M. J. Production of intercellular hormones by mechanical injury. Nature **144**: 553–554. 1939.
122. LUTMAN, B. F. Respiration of potato tubers after injury. Bull. Torr. Bot. Club **53**: 429–455. 1926.
123. MAGER, H. Die Endodermis als Grenze für Stoffwanderungen. Planta **19**: 534–546. 1933.
124. MARSHALL, R. P. The relation of season, of wounding and shellacking to callus formation in tree wounds. U. S. Dept. Agr. Tech. Bul. 246. 1931.
125. MASSART, J. La cicatrisation chez les végétaux. Mém. Cour. Acad. Roy. Sci. Belg. **57**: 2–68. 1898.
126. MENDEL, KURT. The anatomy and histology of the bud-union in *Citrus*. Palestine Jour. Bot. & Hort. Sci. **1**: 13–46. 1936.
127. MERRY, J. Formation of periderm in the endosperm of *Crinum asiaticum*. Papers Mich. Acad. Sci. **22**: 159–164. 1937.
128. MEYEN, F. Phytotomie. 1830.
129. MIRSKAJA, L. Über Regenerationsvorgänge an Vegetationspunkten von *Tradescantia guianensis*. Planta **8**: 27–35. 1929.
130. MOHL, H. VON. Über den Vernarbungsprozess bei der Pflanze. Bot. Zeit. **7**: 641–652. 1849.
131. MOLISCH, H. Zur Kenntniss der Thyllen, nebst Beobachtungen über Wundheilung in der Pflanze. Sitz. Ber. K. AK. Wiss. Wien. I. **97**: 264–299. 1888.
132. ———. Beiträge zur Mikrochemie der Pflanze. Nr. 17. Über ein bei den Kakteen vorkommendes, einen roten Farbstoff bildendes Chromogen. Ber. Deut. Bot. Ges. **46**: 205–211. 1928.
133. MRKOS, O. Über den Einfluss des Wuchsstoffes auf die Regeneration und Wundgewebebildung. Planta **21**: 206–210. 1933.
134. NAKANO, H. Untersuchungen über Kallusbildung und Wundheilung bei Keimpflanzen I. II. Ber. Deut. Bot. Ges. **42**: 261–267; 267–272. 1924.
135. NEEFF, F. Über Zellumlagerung. Zeits. Bot. **6**: 465–547. 1914.
136. NOMMENSEN, R. Beiträge zur Kenntnis der Anatomie der Cacteen, insbesondere ihres Hautgewebes. Diss. Kiel. 1910.
137. NUTMAN, P. S. Studies in vernalization of cereals. VI. The anatomical and cytological evidence for the formation of growth-promoting substances in the developing grain of rye. Ann. Bot. **3**: 731–757. 1939.
138. OLUFSEN, L. Untersuchungen über Wundperidermbildung an Kartoffelknollen. Beih. Bot. Centralbl. **15**: 269–308. 1903.
139. ORSÓS, O. Untersuchungen über die sogenannten Nekrohormone. Protoplasma **26**: 351–371. 1936.
140. ORTH, H. Die Wirkung des Follikelhormons auf die Entwicklung der Pflanze. Zeits. Bot. **27**: 565–607. 1934.
141. PATERSON, A. The occlusion of pruning wounds in Norway spruce (*Picea excelsa*). Ann. Bot. **2**: 681–698. 1938.
142. PETRI, L. Experienze sulla formazione del sughero delle ferrite. Boll. Staz. Patol. Veg. (Florence) **9**: 328–352. 1929.
143. PILKINGTON, M. The regeneration of the stem apex. New Phytol. **28**: 37–53. 1929.
144. POULTER, R. W. Problems of wound healing in red clover stems. Science **90**: 108–109. 1939.
145. REICHE, H. Über Auslösung von Zellteilungen durch Injektion von Gewebesäften und Zelltrümmern. Zeits. Bot. **16**: 241–278. 1924.

146. Riker, A. J. Cytological studies of crown gall tissue. Amer. Jour. Bot. **14**: 25–37. 1927.
147. Rippel, K. Über Teilungs- und Streckungswuchsstoffe. Planta **26**: 164–166. 1937.
148. ————. Zur Frage der pflanzlichen Zellteilungs- und Zellstreckungshormone. Planta **26**: 812–815. 1937.
149. ————. Über den Gehalt von Zellteilungshormonen in Samen und Keimlingen von *Pirus malus, Prunus domestica* und *Prunus avium*. Planta **29**: 1–10. 1938.
150. Roach, W. A. Plant injection as a physiological method. Ann. Bot. **3**: 155–226. 1939.
151. Rodger, E. A. Wound healing in submerged plants. Amer. Mid. Nat. **14**: 704–713. 1933.
152. Rogenhofer, G. Wirkung von Wuchsstoffen auf die Kallusbildung bei Holzstecklingen I. II. Sitz. Ber. AK., Wiss. Wien. Math.-Nat. Kl. I. **145**: 81–99; 179–193. 1936.
153. Ruhland, W., und Ramshorn, K. Zur Steuerung der Pflanzenatmung durch Hormone. Naturwiss. **27**: 391. 1939.
154. Saran, A. B. Note on wounding of the leaves of *Anacardium occidentale* Linn. at different stages of their development and its effect on respiration. Jour. Indian Bot. Soc. **17**: 1–4. 1938.
155. Sass, J. E. Formation of callus knots on apple grafts as related to the histology of the graft union. Bot. Gaz. **94**: 364–380. 1932.
156. Schellenberg, H. Beiträge zur Kenntnis der verholzten Membranen. Jahrb. Wiss. Bot. **29**: 237–266. 1896.
157. Schenk, H. Beiträge zur Biologie und Anatomie der Lianen. 1892.
158. Schilling, E. Über hypertropische und hyperplastische Gewebewucherungen an Sprossachsen verursacht durch Parraffine. Jahrb. Wiss. Bot. **55**: 177–258. 1915.
159. ————. Ein Beitrag zur Physiologie der Verholzung und des Wundreizes. Jahrb. Wiss. Bot. **62**: 528–562. 1923.
160. Schindler, H. Untersuchungen über eine an *Cereus grandiflorus* Mill. beobachtete Gewebenekrose. Ang. Bot. **19**: 505–508. 1937.
161. Schwarz, W. Die Strukturänderungen sprossloser Blattstecklinge und ihre Ursachen. Ein Beitrag zur Kausalanalyse der Gewebebildung. Jahrb. Wiss. Bot. **78**: 92–155. 1933.
162. Seifriz, W. Pathological changes in protoplasm. Protoplasma **32**: 538–550. 1939.
163. Sharples, A., and Gunnery, H. Callus formation in *Hibiscus Rosa-sinensis* L. and *Hevea brasiliensis* Müll. Arg. Ann. Bot. **47**: 827–839. 1933.
164. Simon, S. V. Transplantationsversuche zwischen *Solanum melongena* and *Iresine Lindeni*. Jahrb. Wiss. Bot. **72**: 137–160. 1930.
165. Sinnott, E. W., and Bloch, R. Changes in intercellular relationships during the growth and differentiation of living plant tissues. Amer. Jour. Bot. **26**: 625–634. 1939.
166. ————, and ————. Cytoplasmic behavior during division of vacuolate plant cells. Proc. Nat. Acad. Sci. **26**: 223–227. 1940.
167. Smith, E. F. Mechanism of tumor growth in crown gall. Jour. Agr. Res. **8**: 165–186. 1917.
168. Sorauer, P. Handbuch der Pflanzenkrankheiten. 6. Aufl. **1**. 1933.
169. Spitzer, K. Über das Vorkonnen eines Dioxyphenylderivates und eines spezifischen Fermentes in Apfel und anderen Fruchtarten. Bioch. Zeits. **231**: 309–313. 1931.
170. Steward, F. C., Wright, R., and Berry, W. E. The respiration of cut discs of potato tuber in air and immersed in water, with observations upon surface-volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.

281. WEISS, FREEMAN. 1924. The effect of rust infection upon the water requirement of wheat. Jour. Agr. Res. **27**: 107–118.
282. WELLENSIEK, S. J. 1927. The nature of resistance in *Zeae Mays* L. to *Puccinia sorghi* Schw. Phytopath. **17**: 815–825.
283. WELLHAUSEN, E. J. 1937. Genetics of resistance to bacterial wilt in maize. Ia. Agr. Exp. Sta. Res. Bull. 224.
284. WHETZEL, H. H., ROSENBAUM, J., BRANN, J. W., AND MCCLINTOCK, J. A. 1916. Ginseng diseases and their control. U. S. Dept. Agr. Farmers Bull. 736.
285. WILSON, J. D. 1932. Environmental factors in relation to plant disease and injury: A bibliography. Ohio Agr. Exp. Sta. Tech. Bull. 9.
286. WINGARD, S. A. 1927. The immediate effect of cross-pollination on the size and shape of bean seed. Genetics **12**: 115–124.
287. ———. 1933. The development of rust-resistant beans by hybridization. Va. Agr. Exp. Sta. Tech. Bull. 51.
288. WOLLENWEBER, H. W. 1913. Pilzparasitare Welkekrankheiten der Kulturpflanzen. Ber. Deut. Bot. Ges. **31**: 17–34.
289. WOLF, F. A., DIXON, L. F., MCLEAN, RUTH, AND DARKIS, F. R. 1934. Downy mildew of tobacco. Phytopath. **24**: 337–363.
290. YOUNG, P. A. 1926. Penetration phenomena and facultative parasitism in *Alternaria, Diplodia* and other fungi. Bot. Gaz. **81**: 258–279.
291. YOUNG, V. H., JANSSEN, GEO., AND WARE, J. O. 1932. Cotton wilt studies. Ark. Agr. Exp. Sta. Bull. 272.
292 ———. 1938. Control of cotton wilt and "rust" or potash hunger, by the use of potash-containing fertilizers. Ark. Agr. Exp. Sta. Bull. 358.
293. ZIMMERMAN, A. 1925. Sammelreferate über die Beziehungen zwischen Parasit und Wirtspflanze. II. Die Uredineen. Centbl. Bakt. **65**: 311–418.
294. ZINNSER, HANS. 1914. Infection and resistance.
295. ZOBEL, H. F. 1914. Celery diseases. Gard. Chron. **55**: 95.

WOUND HEALING IN HIGHER PLANTS

ROBERT BLOCH
Yale University

INTRODUCTION

In higher plants, wound tissues formed over artificial or naturally developing injuries are of common occurrence, and their formation has long been described. They usually consist of comparatively simple structures which have attracted the interest of workers in both theoretical and practical botany (108, 168).

The writer feels that in a review discussing the problem of wound healing much emphasis should be laid on those processes which take place in cells and tissues in the immediate neighborhood of the wound, *i.e.*, on the physiological and structural changes which precede and follow the beginning of meristematic activity, including both the phases of degeneration and tissue reorganization. Here the first physical and chemical effects of tissue interruption may be studied, *e.g.*, the degeneration and necrosis of cells, the formative effect of these processes on adjacent healthy cells, and the influence of other external and internal factors on meristem formation and wall differentiation.

Besides superficial formation of scar tissues, more general reactions of a compensating or regenerating character occur, when deep injuries or the removal of embryonic regions interrupt or affect essential tissue and organ communications, or isolate part of the organism. These processes, though related to the problems of wound healing and of great theoretical and practical interest, cannot be discussed at length in this article, particularly as valuable information has become available in recent reviews on tissue and organ cultures, regeneration and vegetative propagation (188, 47, 104, 173).

The majority of mature, vacuolate plant cells, without being separated from the mother organism, are capable of rejuvenation, *i.e.*, they can be induced to divide and to resume growth, though to different degrees in various cell types, and also depending on the location within the plant. Information is thus obtained as regards the potential character of cells, cell layers and tissues at successive stages of development and in relation to each other. In this sense.

171. SWARBRICK, TH. The healing of wounds in woody stems. Jour. Pomol. & Hort. Sci. **5**: 98–114. 1926.
172. SWINGLE, C. F. A physiological study of rooting and callusing in apple and willow. Jour. Agr. Res. **39**: 81–128. 1929.
173. ————. Regeneration and vegetative propagation. Bot. Rev. **6**: 301–355. 1940.
174. TAUBENHAUS, J. J., AND EZEKIEL, W. N. Acid injury of cotton roots. Bot. Gaz. **92**: 430–435. 1931.
175. THORNTON, N. C. Oxygen regulates the dormancy of the potato. Boyce Thompson Inst. Contr. **10**: 339–361. 1939.
176. TIMMEL, H. Über die Bildung anomaler Tracheiden im Phloem. (Beiträge zur experimentellen Anatomie der Wurzel.) Flora **22**: 203–241. 1927.
177. TRÉCUL, A. Accroissement des végétaux dicotylédonés ligneux, reproduction du bois et de l'écorce par le bois décortiqué. Ann. Sci. Nat. Bot., III. **19**: 157–192. 1853.
178. ULRICH, R. Réactions des fruits aux blessures expérimentales. Rev. Gén. Bot. **48**: 354–375; 427–440; 494–504; 539–561; 621–632; 676–694; 725–759. 1936. **49**: 36–65; 124–140. 1937.
179. ————. Mécanisme et conditions de la chute des fruits chez le lierre. (*Hedera Helix* L.) Rev. Gén. Bot. **49**: 601–618. 1937.
180. UMRATH, K., AND SOLTYS, A. Über die Erregungssubstanz der Papilionaceen und ihre zellteilungsauslösende Wirkung. Jahrb. Wiss. Bot. **84**: 276–289. 1936.
181. VÖCHTING, H. Untersuchungen zur experimentellen Anatomie und Pathologie des Pflanzenkörpers. 1908.
182. WARBURG, O. Über den Einfluss der Verholzung auf die Lebensvorgänge des Zellinhaltes. Ber. Deut. Bot. Ges. **11**: 425–441. 1893.
183. WATKINS, G. M., AND WATKINS, M. O. A study of the pathogenic action of *Phymatotrichum omnivorum*. Amer. Jour. Bot. **27**: 251–262. 1940.
184. WEHNELT, B. Untersuchungen über das Wundhormon der Pflanzen. Jahrb. Wiss. Bot. **66**: 773–813. 1927.
185. WEISSENBÖCK, K., AND STERN, A. Wuchsstoffinjektionen bei Pflanzen. Bot. Arch. **39**: 315–331. 1939.
186. WENT, F. W. Auxin, the plant growth-hormone. Bot. Rev. **1**: 162–182. 1935.
187. WERNER, H. O. Wound healing in potatoes (Triumph variety) as influenced by type of injury, nature of initial exposure, and storage conditions. Univ. Neb. Agr. Exp. Sta. Res. Bul. 102. 1938.
188. WHITE, PH. R. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.
189. WIESNER, J. Die Elementarstruktur und das Wachstum der lebenden Substanz. 1892.
190. WILHELM, A. F. Untersuchungen über das Chromogen in *Vicia Faba*. Jahrb. Wiss. Bot. **72**: 203–253. 1930.
191. WILLISON, R. S. Fall spray injury to peach trees. Sci. Agr. **19**: 670–672. 1939.
192. WINKLER, H. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeits. Bot. **8**: 417–531. 1916.
193. WOIT, M. Über Wundreaktionen an Blättern und den anatomischen Bau der Blattminen. Mitt. Deut. Dendr. Ges. **35**: 163–187. 1925.
194. WOODHEAD, N. Studies in growth and differentiation. V. Histological and metabolic changes during wound-healing in *Kleinia articulata* Hand. Ann. Bot. **48**: 467–480. 1934.
195. WRAY, E. M. The structural changes in a woody twig after summer pruning. Proc. Leeds Phil. Soc. Sci. Sect. **2**: 560–570. 1934.
196. WRIGHT, R. C., PEACOCK, W. M., AND WHITEMAN, T. M. Effect on subsequent yields of storing cut seed potatoes at different temperatures and humidities. U. S. Dept. Agr. Tech. Bul. 394. 1934.

197. WYLIE, R. B. Leaf structure and wound response. Science **65**: 45–50. 1927.

198. ———. Cicatrization of foliage leaves. I. Wound responses of certain mesophytic leaves. II. Wound responses of certain broad-leaved evergreens. Bot. Gaz. **90**: 260–278. 1930; **92**: 279–295. 1931.

199. ———. Concerning the conductive capacity of the minor veins of foliage leaves. Amer. Jour. Bot. **25**: 567–572. 1938.

200. ZIMMERMAN, P. W., AND HITCHCOCK, A. E. Adventitious shoots and roots induced by natural influences and synthetic growth substances. Boyce Thompson Inst. Contr. **11**: 127–141. 1940.

THE BOTANICAL REVIEW

Vol. VII — March, 1941 — No. 3

BOTANICAL PROBLEMS IN BOREAL AMERICA. I

HUGH M. RAUP

Arnold Arboretum, Harvard University

CONTENTS

INTRODUCTION

It is the purpose of this paper to outline some of the problems now engaging the attention of botanists working in arctic and subarctic America. To do so without the recital of a certain amount of descriptive matter and the discussion of historical sequence in ideas is well-nigh impossible. Nevertheless, such matter will be reduced as much as seems feasible; and rather than give it separate space under introductory headings, it will be interpolated in appropriate places throughout the paper, with references to pertinent literature.

The geographic boundaries involved must be rather arbitrarily drawn. In common usage the "Arctic Regions" are fairly well defined as the areas north of the limit of trees. A southern boundary formed by the mean isotherm of 10° C. for the warmest month of the year corresponds fairly well with the northern limit of forest growth (Nordenskjold, '28, p. 72). A southern boundary for what we mean by "subarctic" is not so easily defined on a natural basis, and might better be drawn, with our present knowledge, on grounds of botanical convenience. For purposes of exploration and the logical enlargement of botanical knowledge in the northern part of the continent, a useful line may be drawn eastward across central British Columbia, Alberta and Saskatchewan, thence southeasterly through Manitoba and Ontario to include James Bay. From there it follows an indefinite course eastward across the southern part of the Labrador peninsula to the Straits of Belle Isle. It will be noted that this boundary in both the eastern and western sections cuts across natural botanical regions, but in the northern Great Plains it roughly marks the transition from grassland to forest. Its selection is based upon the present extension of our standard manual ranges, and upon the northern limits of country in which we have a fair amount of continuous botanical information. Henry's "Flora of Southern British Columbia" ('15), though far from complete, involves approximately the southern half of that province, and Rydberg's "Flora of the Rocky Mountains and Adjacent Plains" ('22) covers the country north to latitude 55° in British Columbia, Alberta and Saskatchewan. Rydberg's later work on the "Flora of the Prairies and Plains" ('32) extends into southeastern Saskatchewan and southern Manitoba. In eastern Canada comprehensive botanical work has been done only in southern Ontario and southern Quebec. Here our boundary might well be the rather indefinite

northern extent of Marie-Victorin's "Flore Laurentienne" ('35). Although frequent mention of Greenland problems will be found in the paper, no attempt will be made to summarize the work being done there. The reader is referred to the studies of M. P. Porsild ('22, '32), Thorv. Sørensen ('33), Paul Gelting ('34), Devold and Scholander ('33), T. W. Böcher ('33a, '33b) and others for recently published discussions on that region.

Problems in boreal American botany may be divided roughly into three categories dealing, first, with the flora itself, second, with the arrangement of the flora into plant formations and lesser communities, and third, with applied botany. The first two represent two commonly accepted points of departure in the study of plant geography. The first deals with the identity and distribution of the units of plant life which are designated by systematic botanists, while the second involves a study of the flora in terms of landscapes consisting of masses of this or that form of plant life. These two points of view have been kept for too long a time in separate scientific compartments, and their fusion constitutes one of the major philosophic problems confronting modern plant geographers. Most of the earlier students of northern American floras were concerned entirely with individual species, although such men as Sir John Richardson and later the great pioneer Canadian botanist, John Macoun, drew up the major aspects of Canadian plant formations. The analysis and study of plant communities did not begin to acquire its modern form until the late 19th and early 20th centuries; the ideas and techniques for the study of vegetational dynamics and biological life forms were only beginning during that period. Since that time plant geography has been put to a large extent upon a physiological basis. Although a great deal of work on the structure of arctic and subarctic plant communities has been done in the Old World, in America it is hardly more than begun. Botanical work in boreal America during this period of change has remained to a large extent floristic; and geographic problems which have grown out of floristic investigations are still uppermost.

Problems in applied botany deserve more attention than can be given them in the present paper. The aboriginal and modern uses of wild plants, the improvement of native plants by selection, northern agriculture, grazing, and forestry, and the introduction of weeds

or other exotic species, are attractive fields of research that will be mentioned here only by citation.

The discussions in this paper will begin with questions of exploration, physiographic history, and climate; then will follow problems of speciation and endemism, the origin and geographic distribution of the flora, and the development and distribution of plant communities.

The author confesses, at the outset, to a certain amount of personal bias. His own work has been concerned almost entirely with descriptions of reconnaissance field studies in the vascular flora and plant communities of the central and southern parts of the Mackenzie drainage basin. Detailed analytical or experimental studies of communities and speciation have had no part in this except to be drawn upon for inspiration and ideas. It is hardly to be expected, therefore, that equal justice will be done to all the regions of boreal America, or to all phases of the problems involved. Finally, the paper will deal almost entirely with the vascular flora, even though this entails the neglect of many significant questions.

ACKNOWLEDGMENTS

The sources of material, in the realms of both facts and ideas, for such a paper as this become so varied that to give adequate credit to all the individuals who have contributed to it is nearly impossible. Ideas for promising research problems are apt to arise from one's reading, from chance conversations with colleagues or students, or perhaps even from the camp-fire yarning with guides and packers that forms a part of every wilderness collecting trip. Many of these sources are soon forgotten, and often, in spite of their primary significance, are never acknowledged as they deserve. To all of these people, though they must remain unnamed, the writer wishes to express his appreciation.

The following persons have been kind enough to read all or part of the manuscript, and have contributed valued suggestions: Professor M. L. Fernald of the Gray Herbarium, Dr. I. M. Johnston and Dr. A. C. Smith of the Arnold Arboretum, Dr. Ernst C. Abbe of the University of Minnesota, and Mr. J. H. Soper of the Biological Laboratories at Harvard. Suggestions in various special fields have come from Dr. C. F. Brooks on climatic relations, from Dr. Sheldon Reed, Dr. Karl Sax, and Dr. A. O. Dahl on cytology

and genetics, and from Dr. Charles S. Denny on frost action phenomena. To all of these the writer is deeply grateful, at the same time absolving them from responsibility for errors of fact or judgment.

EXPLORATION

No attempt to assemble and publish a comprehensive flora of boreal America has been made for a century. There is no covering manual containing keys and descriptions by which a student may determine specimens from this vast region. The last—and only—catalogue which could claim the distinction of having summarized most of the then existing material was "Flora Boreali-Americana" by W. J. Hooker, published between 1829 and 1840. The nearest approach to it was John Macoun's "Catalogue of Canadian Plants" (1883–1890); but for most of his boreal records Macoun was dependent upon Hooker. Here, then, is the first and one of the most important problems before the botanist who wishes to study northern American plants. He must find his way through the maze of specialized literature that contains a hundred years of fact-finding and opinion; and if he wishes to master even a part of the whole flora he must turn himself at least temporarily into a systematist, and must learn where the gaps are in the body of extant knowledge. The most striking lacunae are in unexplored or partially explored regions.

Probably the largest botanically unexplored parts of North America are in the arctic and subarctic regions. The interiors of the larger land masses of the arctic archipelago and whole segments of the continental area are yet to be explored botanically. In spite of the many expeditions that have been made, for instance, to some of the arctic islands, our knowledge of their flora is still of the sketchiest nature. This can be illustrated by a few figures on Southampton Island. Dr. G. M. Sutton's collection there in 1929–30 increased the known flora of the island from 47 species and varieties to 113 (Raup, '36*a*). Dr. Nicholas Polunin visited it in 1934 and 1936; and utilizing his own collections as well as those of Dr. M. O. Malte who was there in the intervening years (see R. M. Anderson, '35), he increased the list to 171 species, varieties and forms ('38). This makes a total increase in the known flora during the past ten years of over 260%. This performance could be dupli-

cated in many places throughout the arctic archipelago, and the same situation exists in most of the continental interior of the region with which we are dealing. When the writer started collecting vascular plants in the Mackenzie basin there were about 600 species known to occur east of the Rocky Mountains. Very recent figures have not been compiled, but the flora now stands at about 900. By far the greater part of the additions have been made by range extension, but 13 new species and varieties have been described.

Exploration in the past has been very largely circumscribed by the traditional routes of trade and transport; that is, by those rivers and lakes which are known to make fairly easy and continuous routes. This has led to the biological exploration only of narrow strips of territory immediately along the routes of travel. Such a state of affairs led Dr. Charles Camsell, in 1916, to estimate that fully 28% of the whole continental area of Canada remained completely unexplored. Needless to say, most of this was in the northern part of the Dominion. A good illustration of the enormous gaps in our knowledge of plant distribution is to be found in the Mackenzie Mountain region visited by the present writer in 1939. The Mackenzie Mountains are reckoned as the largest single mountain system in Canada. They occupy an area approximately 300 miles wide and 500 to 800 miles long, depending upon how far we carry them into the plateaus to the northwestward. They reach elevations now estimated at some 9,000 to 12,000 feet, and are extremely varied in rock formations and local climates. Nevertheless, except for a few collections made on their southeastern fringes where they come near the Mackenzie River, no plants had ever been collected in them until the summer of 1939.

The number of trained and discerning plant collectors that have gone into boreal America is surprisingly small in view of the great number of specimens that have been brought back. Most of the collections have been made by people who had to sandwich their botanical work into a host of other duties, and who were not especially trained to distinguish different kinds of plants in the field. No stigma can be attached to individual collectors for this. Many of them have made remarkable contributions in spite of handicaps. Nevertheless, the present state of our knowledge of boreal American floras shows all too clearly the effects of the system. It is not unusual to recieve a beautifully selected and preserved collection of

plants from some arctic traveller, perhaps numbering in the hundreds of specimens, but with scarcely a single grass, rush, sedge or willow in the lot. Polunin's recent catalogue of eastern arctic vascular plants ('40) lists 297 species of which over a third (102 spp.) are in these four families. Such a collection, therefore, leaves about a third of the flora totally unrepresented. Polunin lists about 100 collectors who brought plants from his area between 1818 and 1937, with existing specimens totalling at least 50,000 sheets. Perhaps a dozen of these collectors could have been expected to gather gramineous plants and willows as thoroughly as they did other groups, and probably no more than half that number were thoroughly trained botanical field men.

In addition, then, to the acute need for filling in entirely blank spots on the botanical map, there is the necessity for trained collectors in nearly all parts of the north.

Most of boreal America is characterized by vast expanses of absolute wilderness in which there are almost no inhabitants. Transportation facilities are still primitive although time and distance are rapidly being annihilated by air travel. One must go prepared for a much greater degree of independence than is necessary in many of the warmer, more densely populated parts of the earth; for labor is not easy to acquire, and except in a few favored localities, food supplies can be wrested from the country itself only with considerable time and effort. Botanical work must be done during the short open season in which spring, summer and autumn are telescoped. In most parts of the north the mosquito and fly plague must be reckoned with as of prime importance in this summer activity. Those who have not actually experienced the mosquitoes and flies of the northern wilderness can have no conception of the extent to which they condition efficient field study.

In many parts of the world it is feasible to employ good local collectors, thus saving the cost of expensive expeditions. This has never been possible in our region except in a few cases where missionaries or other Europeans with the requisite interest in such matters have become established. The scattered native Indians, living largely by the chase, have never developed a local knowledge of plant life comparable to that found in warmer climates.

The best known part of the arctic archipelago, from a botanical viewpoint, is probably Ellesmere Island. This is due chiefly to the

efforts of H. G. Simmons, botanist to the Second Norwegian "Fram" Expedition (1898–1902). Simmons published a flora of Ellesmereland in 1906; and in 1913 he published a comprehensive survey of the flora and its distribution in the whole archipelago. In addition to his own material, this was based upon miscellaneous collections from the long series of exploring expeditions which, in their search for the elusive northwest passage, had made most of the arctic coast lines well known long before the continental interior had been touched. Simmons' work has remained the most useful account of the American arctic flora until the very recent publication (1940) of Dr. Nicholas Polunin's catalogue of the vascular plants of the Canadian eastern arctic. This catalogue not only brings up to date the listing of available specimens, but also contains an exhaustive survey of both exploratory and taxonomic literature. It includes among many others the materials accumulated from the recent field work of the "Fifth Thule" Expedition (1921–4), of J. D. Soper in Baffin Island (1923–31), of M. O. Malte at various points along the route of the Government Arctic Patrol (1927–8, 1933), of G. M. Sutton on Southhampton Island (1930), of Dr. David Potter on Baffin Island (1937), and of Polunin's own collections from Akpatok Island in 1931 ('34) and on the Eastern Arctic Patrols of 1934 and 1936. The area covered lies north of lat. 60° and east of long. 95°, except for Axel Heiberg Island, Boothia Peninsula and the inland parts of Keewatin.

The eastern Labrador coast has been the object of several expeditions during recent years, and a series of botanical collections have been made and have found their way into herbaria and published papers. Wetmore ('23), Woodworth ('27), Bishop ('30), Abbe ('36, '38) and Potter (see Polunin, '40) have all made contributions in this field, and Abbe has published a rather comprehensive review of phytogeographic problems met with in his work.

The Hudson Bay coast of the Labrador Peninsula is not nearly so well known as the outer coast. Incidental collections by travellers and surveyors are the basis of most of the published botanical information available (see J. M. Macoun, '96). Recent field work, however, the results of which are still unpublished, will undoubtedly improve the situation considerably. Dr. and Mrs. E. C. Abbe spent the season of 1939 at Richmond Gulf where they made large collec-

tions. They also collected at Great Whale River and on the Belcher Islands (Abbe, '39). Material from the Belcher Islands was gathered by J. K. Doutt in the summer of 1938. Collections were made by O'Neill and Dutilly on the west coast of Labrador and on the Ottawa and Sleeper Islands in 1939.

The interior of the peninsula remains almost completely unknown botanically, and is one of the largest gaps in our knowledge of boreal floras. Plants collected in the Lake Melville and Hamilton Inlet regions by R. H. Wetmore in 1921 ('23), and those by Dr. Margaret Doutt on a trip to Grand Falls and Sandgirt Lake in 1939 (unpublished) are among the few specimens available from this vast region. The scanty earlier records were summarized by J. M. Macoun ('96).

The only thorough study of plant communities in eastern arctic Canada of which the writer is aware is that of Polunin on Akpatok Island ('35). A good general description of vegetation in the arctic archipelago and the Labrador peninsula was published by Ekblaw in 1926.

In spite of much travel along the shores of James Bay, only a small part has been thoroughly botanized. With the exception of Dr. David Potter's collections in 1929, most of our knowledge is from a few miscellaneous collections by surveyors. Dr. Doutt has collections made in 1935 on the Twin Islands, Manitounuk Islands, at the mouth of Moose River and at Great Whale River. Potter worked along the southern shores, from Eastmain River to Moose Factory, on Charlton Island, and along Moose River (Potter, '34). His studies on the distribution of estuarine plants on this coast ('32) will be noted in another part of the paper. His publications contain adequate bibliographies of earlier investigations in his district.

The studies of Fr. Marie-Victorin and his colleagues ('35, '38) in southern Quebec lie outside the scope of the present paper, but continued reference will be made to those phases of them that touch upon broader geographic problems. The same may be said of M. L. Fernald's work in Newfoundland and the Maritime Provinces ('11, '18, '21, '24, '25, '26*b*, '29, '31, '33), which has so strongly influenced all thought in boreal phytogeography.

A few localities on the west coast of Hudson Bay have been visited repeatedly, but published accounts of its flora are meager and not up to date except for the portion north of lat. 60°. The latter is

included in Polunin's catalogue ('40). Most of the detailed floristic knowledge available is still in the form of lists published by earlier travellers and surveyors (J. M. Macoun, '11). The southwest coast of the Bay is now easily accessible by rail from Lake Winnepeg, and should very soon become better known. Malte and Polunin have both collected in the Churchill district, which is visited yearly by the Government Arctic Patrol boat; and in 1931 A. E. Porsild worked in that region. Porsild also made extensive collections inland to the west and northwest, as far as the Yathkyed Lake Basin and down Kazan River to Baker Lake (A. E. Porsild, '37*a*). None of these recent collections has been published upon. Johansen ('33; see also Störmer, '33) collected in the Churchill district in 1929; and the members of the Danish Fifth Thule Expedition of 1921–24 made collections there as well as in the interior of Keewatin (See Grøntved, '36). A small collection was made in this region in 1932 by Güssow ('33).

The inland regions south and southwest of Hudson Bay are among the most travelled parts of the Canadian wilderness, yet detailed floristic and vegetational knowledge of them is still lacking. They contain the early trade routes from Hudson Bay into Lake Winnipeg, the Saskatchewan Basin, and the whole northwest country of "Rupert's Land" and "New Caledonia," yet the number of botanical specimens in our herbaria from the whole region of northern Ontario and northern Manitoba (except for the Hudson Bay coast) is trifling. Much of the country is now rather easily reached, either by the railroad northeast from the Pas or by another rail line from Cochrane to Moosonee. The latter was used in 1932 by a party under L. A. Kenoyer who has published a brief survey of plant formations along the route ('40). A great deal of our floristic knowledge of the northern part of the Lake Winnipeg district still rests upon collections of Richardson in 1819–27, of Bourgeau in 1857–9, and of John Macoun. Much of northwestern Manitoba and northern Saskatchewan, although far more accessible than many parts of the north, are almost completely unknown botanically. Recent work at the University of Saskatchewan under Professor W. P. Fraser, and by a few private collectors such as A. J. Breitung at McKague, Saskatchewan, and G. H. Turner at Fort Saskatchewan, bid fair to improve the situation. Prince Albert National

Park in Saskatchewan should be an excellent starting point for botanical survey work in the northern parts of this province.

Hultén has suggested, on evidence derived from a few peculiar plant ranges, "that during the last glaciation a refugium must have existed in the neighborhood of the southern end of Hudson Bay, although not very many species survived on it" ('37*b*, p. 77). No such refuge has been discovered. Other evidence that might point to such a possibility is the occurrence of a group of endemics in northwestern Saskatchewan, to be discussed below.

The only recent general descriptions of western Canadian arctic and subarctic plant communities are those of Preble ('08, '26) and Harshberber ('11). The latter depended largely upon Preble's material gathered in the early 1900's. Preble's remarkable papers ('02, '08) describing his biological reconnaissance in the Hudson Bay region and the Mackenzie basin are indispensable to anyone who contemplates botanical exploration there. They contain large bibliographies and comprehensive digests of all the information on natural features available at the time of their publication.

Until the collections of A. E. Porsild and of the Thule Expedition, already noted, the flora of the interior of Keewatin and eastern Mackenzie was unknown except for a few specimens gathered by surveyors or other travellers. The most important of these were brought back by the geologists, J. B. and J. W. Tyrrell, between 1887 and 1900 (J. B. Tyrrell, '96, '97; J. W. Tyrrell, '98, '01), from their survey routes between Athabaska and Great Slave Lakes on the west and Hudson Bay on the east. A recently published biological survey of the Thelon Game Sanctuary by C. H. D. Clarke ('40), while primarily zoological, contains excellent notes on the general distribution of plant communities. Notes on grazing conditions in the Thelon country, chiefly in connection with the preservation of the musk-oxen, were published by Hoare in 1930. Existing floristic knowledge of such parts of this area as lie in the Lockhart River basin east of Great Slave Lake is included in the present writer's catalogue of plants in the Athabaska-Great Slave Lake region ('36*b*).

Northern Mackenzie, including Great Bear Lake, has been better known than other parts of the northern interior. This is due largely to the fact that some of the early exploring expeditions wintered on Great Bear Lake or in the upper Coppermine region,

and consequently had more opportunity to accumulate botanical material. Most of the western arctic and subarctic material listed in Hooker's classic "Flora Boreali-Americana" came from this region and from the western arctic coast of Canada. The incompleteness of our botanical knowledge of it, however, is attested by the wealth of new facts turned up by A. E. and R. T. Porsild in recent years. These men made extensive collections in Alaska and northwestern Mackenzie between 1926 and 1935 while engaged in reindeer grazing reconnaissance (Porsild, '29). They travelled over much of the country between the Mackenzie and Anderson Rivers and in the Great Bear Lake basin. A. E. Porsild has recently published the results of his Alaskan collecting ('39); and his flora of western arctic Canada is nearly completed. He has also published a useful pamphlet on the edible wild plants of northern Canada ('37*b*). Botanical knowledge of the western arctic coast of Canada was brought up to date by J. M. Macoun ('21), Theodore Holm ('21, '22), and Fritz Johansen ('24) in publications arising from the Canadian Arctic Expedition of 1913–18. More recent material will be included, of course, in Mr. Porsild's forthcoming work.

Excellent gardens have been kept in the Mackenzie basin for many years, some of them dating from the earliest days of the fur trade. Root crops and potatoes do particularly well, though many varieties of vegetables are also successfully grown. Wild hay is abundant in many localities where stock can be wintered without difficulty. Good descriptions of the far northern gardens have been published in recent years by W. D. Albright, of the Beaverlodge Experiment Station ('33*a* & *b*, '37).

The writer began botanical survey work in the Athabaska-Great Slave Lake region in 1926, and has spent, in all, eight seasons in the central and southern parts of the Mackenzie basin. Six of these were devoted to the region immediately around the large lakes, and to the upland country of the Wood Buffalo Park, west of the Slave River. One summer was spent on the Peace River, from the Rocky Mountains to Lake Athabaska, and another in the Mackenzie Mountains west of the Mackenzie River and north of the Liard. The first purpose of this work has been to accumulate material for a floristic study; and the second, to describe and attempt to interpret the plant communities observed. Catalogues of the vascular floras of the

Peace and upper Liard River regions, the Wood Buffalo Park, and the region of the large lakes have been published ('34, '35, '36*b*), along with descriptions of vegetational features in the first two. A discussion of plant communities in the lake country is in preparation. Range conditions in the Wood Buffalo Park have been described in a separate paper (33*b*). The catalogues have included not only the writer's own collections and notes but also those of earlier collectors such as Richardson ('23, '51), Kennicott, Taylor, Preble ('08), Seton ('11) and Tyrrell ('98, '01), in the lake country; and J. M. Macoun, G. M. Dawson ('81), and Mrs. Mary G. Henry in the Peace and upper Liard country. In spite of several field seasons in this region, botanical exploration is hardly more than begun. The situation is summarized in the introduction to the list of plants in the lake country: "As a result of the difficulties of transport, especially in the country which does not lie immediately along the great rivers or lake shores, any detailed studies of natural history are bound to be greatly limited in geographic scope. In attempting generalizations, therefore, on the ranges of species or vegetations, it must be borne in mind constantly that there are vast inland areas which are virtually unknown biologically, and that many of these areas are *terra incognita* as well to the geologist and topographer. With very few exceptions the data upon which the . . . studies have been based were gathered from the marginal strips. The only hinterlands which have been examined with any degree of care are in the Wood Buffalo Park" ('36*b*, p. 180).

It will be seen at once that problems of pure botanical exploration in this region are on every hand. The finding of scattered areas of calcareous and magnesian rocks in the Canadian Shield, with their specialized floras, will add zest to future projects. The isolated or "peninsular" Cretaceous plateaus such as the Birch, Caribou and Horn Mountains, the Buffalo Head, Naylor, and Cameron Hills are almost entirely unexplored, and can be counted on to furnish interesting range extensions and possibly some novelties. There is some evidence that the Caribou Mountains were not so heavily glaciated as the surrounding country.

Large sections of northern Alberta, most of the northern half of British Columbia, and most of the vast wilderness between the Yukon and Mackenzie Rivers are botanical blind spots. The writer's experience in the Mackenzie Mountains has already been

noted, and it must be remembered that only a single locality was visited in the season of 1939. Mrs. Mary G. Henry's collections in northern British Columbia in 1931–1935 ('33, '34) are the largest to date from that region except those of the writer and E. C. Abbe in the Peace River Rockies. A sizeable collection was also made in the upper Peace country by Roy Graham ('34) in 1930. The botany of much of the upper Liard region is known only from the collections made by the geologist, G. M. Dawson, in 1879 and 1887 ('81, '88). The recent clarification of the topographic significance of the "Liard Gap" (Camsell, '36) and its probable biological significance (R. M. Anderson, '37), have rendered this whole district acutely interesting from a biogeographic standpoint. Valuable collections have been made in central Alberta during recent years by A. H. Brinkman and E. H. Moss. The latter has made notable contributions to knowledge of the plant communities of central Alberta, particularly in the region of the parkland and poplar areas (Moss, '32); and together with Lewis and Dowding, has described in detail the bog forests of the region ('28).

There are large parts of boreal America in which there are yet no introduced plants. Noting the first appearance of such plants and tracing their subsequent migrations become fascinating pursuits for botanical observers and collectors. The most valuable contribution to this subject in our region is the work of Mr. Herbert Groh ('37). Some years ago he initiated a weed survey in the recently opened Peace River agricultural district, the plan being to make a census at the end of every five years.

The coastal region of British Columbia is somewhat better known than much of the interior because of the earlier exploring expeditions, but here as elsewhere in the north, thorough collecting will undoubtedly yield a wealth of new material. Dr. G. N. Jones' forthcoming flora of the northwest coast will summarize most of the existing data from this region. Papers by Whitford and Craig ('18) and by Caverhill ('26) contain general descriptions of British Columbia Forests. Strand and dune floras of the Pacific Coast have recently been studied by Cooper ('36).

Early Russian exploring expeditions laid the groundwork for the botany of the Alaskan coast, and it was later enlarged materially by the Harriman Alaskan Expedition. Throughout the long period of exploration, mining and settlement in Alaska, botanical material has

been accumulating, mostly at the National Museum in Washington. Collections by Miss Alice Eastwood and R. S. Williams in the upper Yukon have added substantially. A few of these collections have found their way into print but a comprehensive Alaskan flora has never been published. A. E. Porsild's recent list ('39) is probably the best record to date of the vascular plants of the interior. Dr. Eric Hultén of Lund published an excellent "Flora of the Aleutian Islands" in 1937 ('37*a*) and has begun a general study of the vascular flora of Alaska. He was in this country for several months in 1938 gathering notes and borrowing large numbers of specimens for the project. It is to be hoped that this much-needed addition to our boreal floras will soon be published. Meanwhile specimens continue to come in, especially since the opening of better roads in the interior. What can be done by way of exploration is evidenced by the extraordinary collections of willows by Dr. W. A. Setchell (Ball, '40), and by the material gathered by Miss Edith Scamman ('40).

The most extensive studies of plant communities in Alaska are those of Cooper at Glacier Bay ('23, '31*a*, '31*b*, '37), and of Griggs in the Katmai district ('33, '34*b*, '36). A brief general description of the vegetation has been published by Osgood ('26).

Only recently have the major phytogeographic regions in Alaska begun to emerge. Dr. Hultén (37*b*) has shown that the unglaciated Yukon valley must be contrasted with the surrounding mountain masses and plateaus which were affected by local glaciation. To a large extent these upper levels remain botanically unexplored. Hultén's phytogeographic studies, based in the Bering Sea region, seem destined to have a profound influence upon all of boreal American phytogeography, and will be dealt with in some detail in another part of this paper.

PHYSIOGRAPHIC HISTORY

Boreal America, as defined in this paper, involves an extremely varied arrangement of topographic forms and climates. The outstanding features of this arrangement are fairly well known, and some correlations with the broader aspects of plant life may be drawn at the outset; but physiographic problems arising out of incomplete exploration and hypothetical interpretations of known details render the path of detailed botanical correlations, in both time and space, a thorny one. The significance of the mountain systems in contrast to the broad interior plateaus with all their attendant

modifications of soil, slope and climate, is at once clear as one travels through the region or examines the details of the flora and its distribution. The botanist who selects an area for special reconnaissance or intensive study, however, soon finds himself lost in a maze of problems concerning the origin, age, and stage of development of the land surfaces with which he is dealing. Those parts of the region in which the requisite interpretations have been made by geologists or topographers are so rare and scattered that the botanist either must be content with no physiographic correlations at all, or must become an amateur physiographer and look for his own solutions.

Economic exigencies have limited most of the geological survey work in the north to bedrock. Although this has set the stage for physiographic problems, it has done little toward their solution. The few exceptions to this general rule are mooring stakes to which the botanist can attach himself and acquire a little confidence in his own attempts at interpretation. G. M. Dawson's writings on the superficial geology of the cordilleran and central plains regions ('75, '78, '81*b*, '91), though outmoded in many of their interpretations, contain a mass of botanically useful material. J. B. Tyrrell's works on the Pleistocene history of the Canadian Shield ('96, '97, '98) have formed the geologic basis for many of the broader phytogeographic conclusions of such botanists as Simmons, Holm and Fernald. Likewise, studies of the Pleistocene in eastern Canada by A. P. Coleman ('21, '22, '26), Robert Chalmers ('95, '05), Robert Bell ('63, '84), F. J. Alcock ('24) and others have been drawn upon heavily by Fernald and his students. Short papers on post-glacial lakes and recent sedimentation in the Mackenzie basin by A. E. Cameron ('22) and E. M. Kindle ('18), respectively, have proved invaluable for botanical studies in that region. Capps' studies ('32) of the glacial geology of Alaska will likewise prove useful to botanists working on northwestern phytogeography.

Overshadowing most other considerations in this field is the history of the Pleistocene. The changes wrought not only by the destruction of masses of vegetation, but also by the rearranging of soils, topography and climates are inseparable from the history and present distribution of plants. It will be impossible to discuss the problems involved here in any but the most general terms since, as noted above, they have been reduced to the specific in very few cases. After a brief résumé of the present concepts of the Pleistocene as

they apply to America, the writer will outline some of the questions that have arisen in his own work in the Mackenzie basin.

Dawson refused to accept the thesis of most modern glacial geologists that the surface deposits of the northern Great Plains were laid by a continental ice sheet. He thought the material was brought by ice-rafts in a body of shallow water ('91). Almost no recent student has clung to this view, although Scharff was inclined to doubt the continuity of the great glaciers ('12). An organization of Pleistocene events in boreal America was first set up by J. B. Tyrrell after his extensive travels on the Canadian Shield ('98). Using the bearings of striae and the arrangement of deposited material as data, he concluded that the center of ice formation and spread first appeared in the northern cordilleran region and subsequently in the Keewatin district west of Hudson Bay. Thence it moved southward to a certain extent and later appeared in the Labrador Peninsula where it had a tendency to move northward. By this arrangement, all of boreal America was not covered at once, so that plants could live and move in the west while there was yet ice over the east. Simmons proposed a chronology in the postglacial migration of plants into the arctic archipelago on this basis ('13), and Fernald utilized it in accounting for certain types of disrupted ranges ('25).

Earlier concepts held that the ice sheets were completely destructive over the whole area of boreal America at one time or another; but more recent studies indicate that there were ice-free areas which may have served as refugia during at least parts of the Pleistocene. Such areas have been recognized about the Gulf of St. Lawrence, notably in the Gaspé Peninsula, possibly in the Torngat Mountains of northern Labrador, the well known "driftless area" in Wisconsin, possibly a few localities about the upper Great Lakes, parts of the northern Rocky Mountains and the Yukon Valley, and the northern part of Queen Charlotte Island. Also, it is generally conceded that most of the arctic archipelago was not glaciated. As will be noted in other parts of this paper, the actual availability of some of these areas for plant life during the ice advance is still controversial.

The botanical significance of Pleistocene changes in marine shore lines should also be noted. It is thought that the storage of water in the glaciers exposed large parts of the continental shelf in the arctic, the Bering Sea region, and on our eastern coasts. These lands

could have served as havens of refuge and routes of migration during the maximum expansions of the ice (Hultén, '37*b*). The current studies of Mr. Lincoln Washburn on the ancient shore lines of Victoria Island will doubtless greatly improve our knowledge of changes in that region.

More recent students of American Pleistocene chronology, notably Antevs, have modified the sequence outlined by Tyrrell (Antevs, '25, '28, '34, '36, '38). The effects upon our concepts of floristic and vegetational development are not yet clear in all their details, but the recent work of Hultén will go a long way toward making the necessary readjustment. Hultén's summary of the aspects of current geologic thought that are biologically most significant is as follows:

"To sum up, we know that, since the Tertiary period, at least three, probably four glacial periods, separated by warmer interglacial intervals, have passed over the boreal belt of the globe. There can hardly be any doubt that the glacial periods were synchronous, at least all over the northern hemisphere. . . .

"The extension of the ice-sheets of each separate glaciation is not yet known with accuracy, and it is likewise uncertain whether the ice vanished completely during the interglacials. The extension of the last glaciation in Europe is well known, as also the rate, time and mode of its retreat. Another important feature is also approximately known, *viz.*, the maximal extension of the glaciation. That one warm, long interglacial has existed before the maximal glaciation is also clear. The knowledge of these groups of facts is the most important information we have to rely on in dealing with the problems of the origin and development of the boreal flora" ('37*b*, p. 31). Discussion of Hultén's botanical correlations with these events will be found in the section on the origin and geographic distribution of the flora.

In recent years, Antevs has proposed a modification of the sequence of events in the last, or Wisconsin, glaciation. This is made to include the Iowan period. From a phytogeographic standpoint Antevs' sequence is notable for a late rejuvenation of the Keewatin-Cordilleran ice. The chronological order, according to him, is as follows ('38): "*a*) an early glacial climax of the combined Cordilleran and Keewatin ice sheets west of Hudson Bay and the Great Lakes, called the Iowan; *b*) a general culmination of the Labrador

ice sheet in northeastern North America including the Great Lakes region, usually divided into the Tazewell and Carey substages; *c*) a climax of the Patrician ice sheet in Wisconsin and Minnesota, called the Early Mankato; and *d*) a rejuvenation and second culmination of the Cordilleran and Keewatin ice sheets in northwestern North America, called the Late Mankato. Contemporaneously with the Iowan and Late Mankato there were glacial climaxes in the mountains of western United States called the Tahoe and Tioga glaciations in the Sierra Nevada".

Studies of flora and plant communities in the central part of the Mackenzie basin give considerable evidence that the plant cover is exceedingly young (Raup, '30, '33*a*). Much of the vegetation looks as though it were in the process of being formed. It has always been difficult to reconcile this with the theory of a regular progression of glacial episodes from west to east, so that a late rejuvenation of the ice in the Keewatin district is especially acceptable.

Of scarcely less importance than the actual removal and deposition of material by the ice, at least in the Mackenzie basin, are the post-glacial lakes which covered enormous sections of the country. Normal drainage to the north was stopped by barriers of ice or till so that the waters of such major streams as the Athabaska, Peace and Liard Rivers were impounded to great depths. The ancient shore lines of these lakes were noted years ago by the Canadian geologists. They were named "hyper-glacial" lakes by J. B. Tyrrell ('96) who found them to be of wide occurrence in parts of the Canadian Shield. Cameron has described a series of them in the Athabaska-Great Slave Lake country ('22), having shore lines up to 1600 ft. above sea-level (the present level of Lake Athabaska is about 590 ft.). Rutherford has described broad expanses of glacio-lacustrine soils at elevations of 2000 ft. or more in the Peace River agricultural region ('30).

In spite of the long time since they were first recognized, we have only the haziest ideas on the extent of these lakes. Even allowing for a certain amount of isostatic readjustment of surfaces after the retreat of the ice, one needs only to examine a map showing the general topography of the region to recognize their significance in post-glacial affairs. We know still less about how long they lasted. Rutherford states that even the higher ones, in the Peace River region, remained long enough to accumulate "upwards of 200 feet

in places of fine textured muds". At any rate, the history of plant life in a large part, if not most, of the southern two-thirds of the Mackenzie basin must be reckoned as "post-lacustrine" rather than merely post-glacial. The writer has suggested elsewhere ('34) that similar conditions probably occur in the Winnipeg basin and in the valleys and plains drained by its western tributaries.

The lakes did not all drain off gradually, for definite systems of shore lines can be recognized on the slopes of the Cretaceous plateau remnants that dot the region. There is some evidence that the lacustrine soils of the lower surfaces have not developed their profiles so far as those at 2000 feet (Raup, '34). Plant life could invade the region, therefore, by stages, the duration and dating of which are quite unknown. Furthermore, we do not know whether the process is still going on. Great Bear, Great Slave, and Athabaska lakes are of course lined with ancient shores, the higher ones reaching hundreds of feet above the present levels and the lower ones in a continuous series with the modern beaches. No one has ever examined these shores, many of which could be traced for many miles, for evidence of crustal readjustment. Bent water planes have been detected around the Great Lakes, and probably occur also in the north.

There is some evidence that permanent changes of level have occurred within historic times or have become a part of aboriginal legend. When Mackenzie set out from Chipewyan in 1792 on his journey up the Peace River he went into the Peace by way of Lake Claire and Pine River, a route through the delta which has been out of use for many years. In fact, there is now no water connection between Lake Claire (in the Peace-Athabaska delta) and the Peace River, which would indicate considerable shifting of water levels since Mackenzie's time. Anyone using the route now would have to make one or more portages, but Mackenzie mentions no portage, nor is there one shown on his map. It is possible that this change is due to sedimentation and shifting of channels, but it could also be effected by a lowering of the outlets below.

King's narrative of his travels through the Lockhart basin ('36) contains an Indian legend to the effect that Great Slave and Artillery Lakes were once connected by a narrow strait with gentle current, and that the change to present conditions occurred rather suddenly. Ross reported a somewhat similar legend applied to the western end

of Great Slave Lake, where the opening of the Mackenzie River and the drainage of a larger lake was recounted as occurring cataclismically ('62). Critchell-Bullock ('30) records a conversation with an Indian at Stony Island, Great Slave Lake, in which he was told "that fifty years or so ago it was possible to pass through the centre of the island by canoe. The centre now, however, stands at a height of several feet above the surface of the surrounding lake". J. W. Tyrrell's map of the eastern end of Great Slave Lake ('01) shows a part of the distal end of Fairchild Point as an island, though it is now joined to the peninsula by rocky ledges several feet high.

Mention should be made of some local physiographic problems that are closely related to current botanical research. The development of plant communities on the shores of the larger lakes is conditioned by what appear to be periodic changes in lake levels, and by the phenomenon of ice-push. Although changes in level are known to occur, their periodicity remains to be investigated. Needless to say, an understanding of such changes is essential before shore-line successions can be worked out.

Ice-push is almost universal on the shores of the larger lakes except in protected embayments. The theories that have been propounded to explain its effects are adequately summarized by Scott ('26). For our lakes the observations of J. B. Tyrrell ('10*a*) indicate that the direct effects of expansion and contraction in the ice mass during the winter are of no consequence, and that the work on the shores is done during the spring break-up. As the ice melts it disappears first from the shores, leaving a lane of open water surrounding a central mass. This great block of ice is then blown this way and that on the lake, at the will of ordinary winds. It strikes one shore after another with prodigious force, driving even the heavier shore materials into ridges. Great boulders are often forced inland many yards, sometimes plowing a channel where they are shoved along. After the final disappearance of the ice the ridges which have been pushed up are left standing, or are modified by subsequent wave or wind action, depending upon the nature of their materials. If the materials are of large fragments of rock the ridges are apt to remain intact, but if they are of sand they will be worn down by subaerial erosion to only a fraction of their original size.

To work out the history of the vegetation on any given shore, therefore, it is necessary to know in what period it stands in the

cycle, if there be a cycle, of changes in level; and to know how much of the existing formation of the shore is due to be eliminated by the next rise in level, or by winds, waves and ice-push.

There are great ridge systems composed of sand or of heavy rock fragments on the south side of Lake Athabaska which appear too large to have been formed by ice-push. Ridges of heavy, angular rocks have been observed near Poplar Pt. which are 20 or more feet high and of regular contours. They are roughly parallel to the present shore system and appear to be topographically related to it. It may be that they are part of some morainic system, but the rocks do not look to be of glacial origin. The high sand ridges which margin the shore on either side of William Pt. constitute another enigma. They are much too high and massive to have been pushed up by lake ice. It is suggested either that they are barrier beaches formed off shore entirely by wave action during a long period of emergence, or that they are barrier beaches which have in some way been accentuated by ice-push. We cannot hope to understand the development of plant life on these anomalous shore forms until far more is known of their history.

The sand dune country south of Lake Athabaska has never been examined by a physiographer, and was scarcely known to exist until the last few years. In one area alone, south of William Pt., there are about 200 square miles of open shifting dunes. So far as is known, all of the moving sand is at or near the present shore of the lake; but fixed, or fossilized dunes may be seen far inland. The sand is derived from a pre-Cambrian sandstone which is widespread in the upland country south of the lake, but how far inland the ancient dunes are to be found is still conjectural. It is not impossible that a precarious plant habitat consisting of shifting sand has existed on the south side of "Hyper-Athabaska Lake" for a very long period, moving northward as the actual shore of the lake receded in that direction. A rational explanation of the development of plant communities in the sandy country awaits some sort of description of its history in post-glacial time. The problem is accentuated by the fact that the modern dunes harbor a group of distinctive plants, some of them apparently endemic (Raup, '36*b*).

A brief discussion of pond floras in the Athabaska-Great Slave Lake region will be found in another part of this paper, but it should be noted here that the problems of origin and arrangement of the

ponds are basic considerations. The actual number of small bodies of water, especially on the Canadian Shield, is almost incredible. This is now being realized fully for the first time, due to recent aerial photographic mapping. A glance at the new maps (Nat'l Topog. Series) will show that the ponds are arranged in patterns and series which are apparently correlated with old drainage systems and rock formations not entirely obliterated by the Pleistocene ice. If we superimpose upon these patterns a vertical dimension which will involve periods of time during which the larger lakes have been receding from parts of the region, we get a series of problems in the sequence of development among pond habitats. This is an open field for investigation. The writer has toyed with it to a certain extent in some of the upland portions of the Wood Buffalo Park ('35, pp. 12–14, 41), but the pre-Cambrian country, in which the lake systems are much more highly developed, has not been touched.

Finally, some note should be made of the Cretaceous plateaus that lie east of the northern Rocky Mountains. J. A. Allan could find no evidence of glaciation on the tops of the Swan Hills south of Lesser Slave Lake ('19), and the present writer has found soils on the eastern edge of the Caribou Mountain Plateau which appear to have been weathered *in situ* from the underlying rocks ('35). If these areas and others like them can be proved to have come unscathed through at least the later episodes of the Pleistocene, then they are in the category of possible refugia for plant life, and should be examined with care for any light they may throw upon the distribution of the flora. Many of the more southern plateaus are now easily accessible, but more northern ones such as the Cameron Hills, the Horn Mountains, and parts of the Caribou Mountains can be reached easily only by air.

The phases of physiographic change having to do with ground frost will be found in the discussion of plant communities.

CLIMATE

For discussion of the broader aspects of boreal American climates the reader is referred to three comparatively recent works. The first is "The Climates of North America," by Ward and Brooks ('36); and the second, "The Canadian Climate" by C. E. Koeppe ('31). Ward and Brooks' paper deals with all of the continent except Canada, which is the subject of a later publication in the same series by the Canadian climatologist, A. J. Connor ('38).

Although Canada is not treated in detail by Ward and Brooks, a series of general climatic maps for all of the continent are contained in their paper. These maps were prepared with the collaboration of Connor, and have also been published separately (Brooks and Connor, '36). Koeppe's book is concerned entirely with Canadian climate, and is based upon a large mass of data gleaned from many sources.

There is no occasion here for descriptions of known climatic phenomena, either general or local, in our region. The climates of various parts of the area have been noted in more or less detail by various writers, and are readily available. The following may be consulted in this connection: Labrador Coast, Abbe ('36); Gulf of St. Lawrence region, Marie-Victorin ('35) and Fernald ('25, '33); arctic archipelago, Simmons ('13); Canadian eastern arctic (general), Middleton ('35); Keewatin and northeastern Mackenzie, Blanchet ('30); Manitoba, Connor ('39); northern Mackenzie and parts of Keewatin, Porsild ('29, '37*a*); Thelon Game Sanctuary, Keewatin and Mackenzie, Clarke ('40); Wood Buffalo Park and Peace River regions, Raup ('34, '35); Western arctic Canada, Connor ('37); arctic regions (general) Nordenskjold ('28) and Mecking ('28); arctic Canada (general), Stupart ('28); Alaska, Edith M. Fitton, in Ward and Brooks ('36). This is by no means a complete list, but it will serve as a nucleus for reference.

The scarcity and discontinuity of meteorological data from most parts of the north are the chief difficulties in the way of attempted botanical correlations with climate. There are a few old settlements along the main routes of travel in which records have been kept for many years; but there has been little uniformity either within the stations themselves or from one to another. Large centers of population or extensive agricultural developments which would have encouraged better meteorological observation are rare or non-existent. In the Peace River country, where agriculture has been enlarging rapidly during the past 30 years, the writer found great difficulty in assembling comparable data from seven stations over a continuous period of ten years. For a large part of boreal America there are no climatic data whatever, or only the isolated observations of travellers or temporary residents. The geographic botanist, therefore, must be content with whatever outline of general climatic conditions can be pieced together from records at the nearest sta-

tions, and with the meager observations he can make for himself or get from other travellers before him.

Effective methods for the study of local climates, even for short time intervals, have been greatly improved in recent years (Brooks, '31). An aneroid barometer, sling psychrometer, maximum and minimum thermometer, and pocket thermometer will yield a surprising amount of useful information about local conditions, particularly if two or more members of a field party can work together, making simultaneous records. For instance, the writer's max. and min. thermometer was kept in operation at Brintnell Lake, in the Mackenzie Mountains from June 17 to August 20. It was in open spruce forest at the shore of the lake, surrounded by high mountainous country. The maximum temperatures remained consistently low during the whole summer, rising above 80° F. on only 3 days. For days on end it remained in the 60's, with but little diurnal fluctuation. New snow appeared above timber line as early as the 4th of August. But at no time during the summer did the minimum go below 32°, and it reached this point only once, on Aug. 6th. These figures suggest a cool, but equable climate in summer, and may prove significant in accounting for a local, relatively rich Canadian forest in an otherwise decidedly arctic situation. Records made at Simpson, 200 miles to the eastward, during the same period showed a much more continental climate with larger diurnal fluctuations.

Frost data, as such, are not available for most of the region. To arrive at figures for the average frostless season one must rely upon the recorded minima which may or may not indicate effective frost. The town of Chipewyan on Lake Athabaska is situated on a long, gentle slope along the shore. The weather records have been kept for many years by the Hudson's Bay Company traders, the Anglican Missionaries, or the resident Government Doctors, all of whom have been situated near the upper end of the slope. Gardens at the lower end show the effects of killing frosts in the autumn long before they do so around the weather station. Evaporation data are practically non-existent for nearly all of the region.

Certain isolated areas are known to have remarkably warm summer climates, with an unfailing frostless season of considerable length. Some of these, situated on the shores of large lakes, may be due to a local ameliorating influence of the large bodies of water

(Raup, '35). Others, such as the Vermilion region on the lower Peace River are not so situated, yet they have been known as garden spots for many years. Still others, such as McMurray on the lower Athabaska River, are known to have extremely short growing seasons, though farther south. Similar local variations are common throughout boreal America, especially in regions of high relief; but their phytogeographic significance will be recognized only after a great deal more exploration has been accomplished.

Several phases of the relations of northern plants to climate that have already been the subject of much investigation will only be mentioned here, though further discussion of some of them will be found in other parts of the paper. The ultimate causes for nanism and other structural adjustments still present challenging problems, particularly as they may be related to genetic selection among the plants. The relation of climate to the incidence of polyploidy and genetically increased vigor is now the subject of considerable research, and should throw light on many problems of boreal speciation and distribution. The telescoping of the northern seasons has been described repeatedly, but the actual mechanisms by which northern species manage to get through their reproductive cycles in such short periods are still obscure. A light climate involving continuous daylight during the growing season must involve physiological arrangements within the species quite unknown in temperate regions. Porsild ('37*a*) has recorded that *Saxifraga oppositifolia, Draba alpina* and *Papaver radicatum* in the American arctic will begin their season's growth, flower, and mature their seed, all in 30 days.

One of the most notable features of the arctic and subarctic regions is the slow rate of organic decay. Bacterial action is extremely slow, and consequently the sources of available nitrates as well as other salts are meager. Griggs has discussed this problem both in his studies of the Katmai volcanic region ('33) and in his notes on tundra vegetation ('34*a*). Polunin likewise has noted the deficiency of available nitrogen in the Akpatok habitats ('34–'35). Plants that inhabit cold soils containing much raw humus must get along with the limited supply of soil nitrogen available, or they must have a way to utilize atmospheric nitrogen. The adjustments of northern plants to this end constitute a rich field of investigation. The present writer has observed the abundance of ray fungi, or "Strahlen-

pilze" in some northern soils. One of the more conspicuous of these, forming "knobs" on the roots of alders, is known to fix atmospheric nitrogen like the root nodules of the Leguminosae. They are also known to occur with the Elaeagnaceae, of which *Shepherdia* is extremely common in the north. The extent to which these organisms may go in solving the nitrogen problem for northern floras is unknown.

ORIGIN AND DISTRIBUTION OF THE FLORA

Speciation and Endemism

No estimates of the total number of species in boreal America are at hand, but in general the flora is a relatively small one. There are probably not over a thousand species of vascular plants in the Mackenzie drainage basin east of the Rocky Mountains, and Polunin lists only 297 species in all of eastern arctic Canada north of lat. 60°. Hultén counts 481 species on the Aleutian Islands and the western part of the Alaska Peninsula. Porsild lists 648 in his Alaskan collections, and estimates 750 in the Northwest Territories west of Hudson Bay. The number of native species in the range of Gray's Manual (7th ed.) is 3413.

The numerical distribution of species within the families shows a somewhat higher percentage of grasses and grass-like plants (Gramineae, Cyperaceae, Juncaceae) than in temperate or tropical countries. Within the range of Gray's Manual (7th ed.), for instance, about 20% of the native flora are in these families, while in Polunin's area nearly 30%, and in western arctic Canada (Porsild, '37*a*) about 25% are to be so classified.

Of far greater present significance than the above figures are the "quality" of boreal American species and their often discontinuous distribution. Many of our most knotty problems turn upon the fact that the plants show varying degrees of specific segregation, and that there are patterns in the geographic distribution of segregation. A large percentage of the boreal flora of America consists of what might be termed "fluid" species, with poorly defined structural discontinuities between closely related ones. Any one who has struggled with the determination of northern specimens in such genera as *Potamogeton, Bromus, Poa, Agropyron, Elymus, Calamagrostis,* several species complexes in *Carex* and *Juncus,* not to mention *Salix, Rosa, Potentilla* and many other dicotyledons, will understand the meaning of the term "fluid." Polunin, in describing

his troubles with the extremely polymorphous *Poa glauca,* has illustrated the whole problem: "This is a veritable *typus polymorphus,* of which, concerning material from Ellesmere alone, Simmons wrote 'I have had a good deal of trouble in coming to a conclusion as to how my material was to be arranged, and only with hesitation I place here two of the forms mentioned below.' Hence, with abundant material from almost all over the Eastern Arctic, I had few misgivings about abandoning my early attempts at sorting it all according to the various major tendencies exhibited. To be sure, several described 'varieties' were represented in this material; but transition forms were so abundant and the characters were so unstable that the task seemed futile, and I soon came to agree with Porsild . . . that *Poa glauca* 'varies without limits according to the quality of the place' " ('40, pp. 67–8). This state of affairs is surprisingly general throughout the flora, and seems to be increasingly marked as more material accumulates.

Fernald has outlined the distribution of specific segregation in eastern North America ('31), showing that the "best" species are in the forests of the Appalachian plateaus south of the limits of Pleistocene glaciation, while the poorly defined ones are most abundant on the young lands that were denuded of vegetation by the last ice. Within the boreal region the areas that show the sharpest definition among species are in parts of Alaska, the northern cordillera, and some limited portions of the country around the Gulf of St. Lawrence.

In general, the flora of boreal America is poor in local endemics, and such as occur are not evenly distributed. They follow the geographic pattern of clearer segregation noted above, and are commonest in the western mountains and the Bering Sea region. There is a considerable number about the Gulf of St. Lawrence, a few in the Torngat Mountains of Labrador, and a few in the region of Lake Athabaska.

It should be noted that the recognition of actual endemism in large parts of our area is not easy, due to the large gaps in floristic knowledge. The writer's own experience in the Athabaska-Great Slave Lake region will illustrate. After six summer's botanizing in various parts of that region only two species had been described as new to science; but in the seventh season the sand dune country south of Lake Athabaska was visited for the first time, and yielded no less than 12 new species and varieties. We do not know how wide a

range these novelties will prove to have, since so much of the surrounding country is botanically unexplored. Two of the species described, *Euphrasia subarctica* and *Cicuta mackenzieana,* have proved to range rather widely in the subarctic forest regions. The *Cicuta* has been found as far east as Churchill, and the *Euphrasia* appears to be identical with plants collected in Alaska. Another, *Deschampsia mackenzieana,* seems to be a segregate from a Bering Sea complex (Hultén, '37*b*, p. 108) ; but all of the others appear to be local. They vary from what are designated as regional varieties in wide-spread boreal groups such as *Salix brachycarpa* var. *psammophila* in the *glauca* complex of willows, to extremely well segregated species such as *Salix Turnorii. Salix Turnorii* is a beautiful willow growing among the sand dunes and having a rather tall, columnar habit. Its bark is gray-green in color and its leaves are small, regularly glandular-serrate, and smooth. It has bright red young twigs and smooth, pedicelled, reddish-brown capsules. It is so distinctive in a whole series of essential characters that it does not seem possible to place it logically in any of the commonly accepted sections of the genus. The same is true of a species of *Armeria* (*Statice*) described from the same area. This plant is considerably more robust than most American arctic material of the genus, and has distinctly broader leaves. American species of *Armeria* have been defined largely on the basis of the character of the pubescence on the calyx, but the Athabaska Lake plant proved to have no pubescence at all on the calyx, thus eliminating it from all sections of the genus known to occur in America.

Thus we have not only the problem of the occurrence and authenticity of endemic forms in the midst of a region otherwise known to be very poor in endemics, but we also have to account for a wide variation in the degree of what specific segregation there is.

The nature of specific segregation among many boreal plants, as well as the existence of a pattern of distribution is suggested by the detailed study of certain large species complexes. This can be illustrated with *Salix glauca* and its relatives as they are known to occur in North America. *Salix glauca* is a shrub usually from 2 to 6 feet high, with pubescent twigs, and ovate or obovate leaves which are green above but distinctly paler and glaucous beneath. The leaves are usually pubescent or woolly, especially beneath, at least when young. The catkins come out with the leaves, and are borne on short, leafy peduncles ; the capsules are woolly, borne on short pedi-

cels, and subtended by bracts that are persistent and usually yellowish throughout. This description will cover fairly well some eighteen or twenty different willows in the American flora, to say nothing of the Eurasian. There has been considerable controversy as to whether typical *S. glauca* occurs at all in America (Raup, '31). It was described from Lapland by Linnaeus, and is apparently extremely variable in northern Eurasia as it is in America.

Authentic, or near authentic *Salix glauca* is not known to occur in northeastern America. It is limited to western arctic and subarctic Canada, and to the northern cordillera. On the basis of leaf shape and pubescence, this near-authentic population can be divided into four separate varieties with moderately well defined geographic areas (vars. *glabrescens, acutifolia, poliophylla, ovalifolia*). A doubtful segregate in the central Mackenzie basin, *S. desertorum,* probably belongs here. In the eastern arctic and on the eastern mountains is a group of forms which has been set off as a separate species, *Salix cordifolia.* This looks very much like *glauca* except that it has its leaves much shortened and rounded, even to heart-shaped at the base. Several local varieties and forms of this segregate have been described (Fernald, '26*a*). In Greenland is a related short-leaved form called *Salix anamesa.* The aments of the *cordifolia* group look very much like those of true *glauca.* Then we come to a series primarily confined to the Rocky Mountains, but extending eastward to Hudson Bay, some of them with outliers in the Gaspé. This series, probably involving two species complexes, *Salix brachycarpa* and *S. pseudolapponum,* is distinguished by its short aments. They are short-oblong to spherical in shape. *Salix brachycarpa* has been subdivided into four separate entities, mostly on the basis of growth habit or pubescence. Another group of *glauca* relatives, all arctic or subarctic, is distinguished by having very short petioles to the leaves. In the east are *S. fullertonensis* and *S. chlorolepis,* while in the western arctic are *S. niphoclada* and *S. lingulata.* It should be noted that all of these four are rather poorly defined, and seem to be distinguished by a somewhat fastigiate habit of growth. The greatest divergence from *S. glauca* is shown by *Salix MacCalliana,* a handsome Rocky Mountain and northern Great Plains species. It has smooth, rather leathery leaves that are distinctly dentate.[1]

[1] The nomenclature of this group of willows is far from clear. The names used here, with some modifications, are those settled upon by Schneider in his monographic studies of American willows ('21).

There is no ready explanation for this arrangement of segregation, but it is apparent that the "best" segregates are in the Rocky Mountains and in the Gulf of St. Lawrence region. These are represented by *S. brachycarpa* and *S. pseudolapponum* in the west, and by *S. cordifolia* in the east, all with their various subdivisions. Most of the poorer segregates are in the intervening arctic and subarctic country, and are set off by such variable characters as growth habit, the length of petioles, and the character of the pubescence.

Mention should be made of some recent research in cytogenetics that is pertinent here, though a comprehensive discussion of the mechanism of speciation is impossible in this paper. It has been shown that the distribution of polyploidy within certain species and groups of species has a geographic aspect. Anderson, for instance, has found that in *Tradescantia virginica* and its relatives there are diploid and tetraploid races which "are morphologically indistinguishable but when separated by cytological means it is found that the tetraploids are, on the whole, a little larger, considerably stouter, a little longer-flowering, a little hardier, and a little easier to grow under varying conditions. That these differences operate in nature and are of taxonomic consequence is shown when the distribution of the diploids is compared with that of the tetraploids. The diploid species are of limited distribution and even in those areas where they do occur are usually restricted to one particular habitat. By contrast, the tetraploid species and races have wide distributions and most of them have the ability to flourish under a variety of situations" ('37, pp. 336–7). Manton has shown that diploid and tetraploid races of a certain crucifer in Europe occupy, respectively, isolated, ancient habitats at low elevations, and youthful alpine habitats which were covered by the alpine glaciers ('32). In similar vein, Hagerup has demonstrated that polyploidy is highly developed in such "unfavorable" regions as the cold arctic, or in extremely hot regions ('32). Stebbins ('40, pp. 62–3) has summarized much of this as follows: "One important generalization which undoubtedly holds good for the bulk of polyploid complexes, including *Crepis* and *Tradescantia,* is that the polyploids are dominant in regions which have been only recently opened to occupation by plants, or which have been subjected to great climatic or other environmental changes, while the diploids tend to occupy the older, more stable habitats. This makes the study of polyploid complexes very important from the standpoint of plant geography."

A recent paper by Bowden ('40) gives the results of a study of possible correlations between winter hardiness and polyploidy. This author concludes that no good correlation exists. Clausen, Keck and Heisey ('40, p. 423) reached the following conclusion from their transplant studies in California: "Until very recently an opinion has been rather widespread that in polyploid complexes, forms with the higher chromosome numbers occupy the more adverse environments. Recent literature, however, is replete with examples that substantiate the observations made in the present investigations that there is no support for this view."

The extent to which these findings can be applied to boreal problems of speciation and distribution is yet to be discovered. It should be noted that polyploidy is not universally spread among the families of plants. In some prominent northern groups like the Gramineae and Rosaceae it is of common occurrence, while in others such as the Cyperaceae and Juncaceae it is obscured, according to Stebbins ('40), "by the large number of aneuploid or non-multiple series found in them."

Further discussion of genetic investigations significant to these problems will be reserved to the treatment of Hultén's hypotheses concerning distribution.

For recent reviews of literature and thought on problems of speciation the reader is referred to such papers as Anderson's "Cytology in Its Relation to Taxonomy" ('37), Du Rietz's "The Fundamental Units of Biological Taxonomy" ('30), Faegri's "Some Fundamental Problems of Taxonomy and Genetics" ('37*a*), Dobzhansky's "Genetics and the Origin of Species" ('37), and Stebbins' "The Significance of Polyploidy in Plant Evolution" ('40).

The Theory of Persistence

The Darwin-Hooker Concept. The origin and distribution of the flora in boreal America have been the subjects of long series of investigations and published papers. The arctic flora in particular has been a battle ground of theory and opinion for generations. As in so many other fields of biology, Darwin's "Origin of Species" supplied new impetus to the study of plant geography. It began what may be termed the first phase of modern floristic phytogeographic theory. A second or recent phase did not begin until 1925, with the publication of M. L. Fernald's paper on the "Persistence of Plants in Unglaciated Areas in Boreal America" ('25).

J. D. Hooker's "Outlines of Distribution of Arctic Plants," published in 1862, was the first detailed effort to apply to a boreal problem the ideas laid down in Darwin's two chapters on geographic distribution. Hooker had at his command a considerable volume of collected material with which to work, but due to the incomplete exploration of so much of the American and Asiatic arctic, his conclusions were bound to be heavily weighted in favor of the much better known arctic parts of Europe.

Following Darwin, the burden of Hooker's argument was that during the glacial period the arctic floras were forced to migrate southward to more suitable climates. After the retreat of the ice these arctic plants all returned northward except for some which found their way to the tops of mountains where the growing conditions were similar to those of the far north. Here they became isolated from their kind by great distances and altitudes, thus giving rise to their existing disrupted ranges. He divided the arctic into several subdivisions: first, the Greenland district, which he considered to be almost exclusively "Lapponian" in its floristic affinities; second, the arctic European district, extending eastward to the Obi River and including Novaya Zemlya and Spitzbergen; third, the Asiatic district; fourth, a west American district extending from Bering Straits to the delta of the Mackenzie River; and finally, the eastern American district. He recognized a circumpolar element in the arctic flora and separated elements related to the separate geographical areas, but his too broad definitions of the arctic Scandinavian led him to consider the northern European affinity to be the most important.

The first notable contribution to the botany of the American arctic after Hooker was that of H. G. Simmons in 1913. Not only an increased amount of botanical information was now available, on both arctic and alpine floras, but also a great many geological investigations had contributed to a wider knowledge of the all important influence of the Pleistocene glaciation. It was particularly significant from a phytogeographic standpoint that ice-free lands should be defined in other areas margining the great continental ice sheets beside those to the south. Simmons also made use of the relative amounts of endemism in the arctic as a criterion for the age of the flora. One of the outstanding facts concerning the flora of the American arctic archipelago is its very low percentage of ende-

mism. He took issue with Hooker as to the Scandinavian origin of the flora, and maintained that northern Scandinavia, if truly arctic species only were counted, would prove to have a very poor representation of them in comparison to other parts of the arctic.

Simmons considered that the ancestors of the arctic flora were an ancient group, probably living in the far north during the Tertiary. Although he cites evidence that during the Pleistocene most of the arctic archipelago was unglaciated, he did not believe that the arctic flora could have lived there during the glaciation due to the rigorous climate. He considered, further, that the pre-glacial flora was very uniform throughout in species composition. It was forced to migrate southward or was destroyed during the maximum advance of the ice. With the disappearance of the ice it wandered northward again to reoccupy its old territory, leaving isolated alpine remnants, but taking with it some typically alpine species which were more or less limited to the longitudes in which they had originated. These "longitudinal contingents," as he termed them, were largest in America and smallest in Europe. Greenland, under this scheme, remained an enigma, since it possesses a large American element and also a considerable number of European species.

Simmons considered that the most important refuge for arctic plants during the Pleistocene was west of the Mackenzie River, where glaciation was not so widespread. Following the analysis of Tyrrell ('98), he adjusted the reimmigration of plants to the arctic archipelago to the theory of the progressive advance of glaciation from west to east. He therefore thought that the archipelago got its first post-glacial vegetation by way of the arctic coast, and Banks and Victoria Islands. At this time the center of the ice cap would have moved southeastward so as to free the northern coast of the continent. As this went on, the next route of migration which was opened to plants from the west was by way of King William land and the peninsula of Boothia Felix. Later, a third element could utilize the west coast of Hudson Bay, and could draw upon such plants as had found refuge in the eastern part of the continent; and with the final disappearance of the Labrador ice sheet, plants began to migrate northward through the Labrador Peninsula. Some of these plants he thought might have moved northward during a warm period in post-Pleistocene time. The small amount of endemism in the arctic archipelago he used as argument against the

possibility of plants having persisted in place during the Pleistocene.

Like Simmons, Theodore Holm recognized northern and southern elements in the flora of the American arctic ('22). He was concerned mainly with the plants of the western continental rim collected by Johansen on the Canadian Arctic Expedition of 1913–18. In general, his concept was the classic one that the glaciation had driven southward an American arctic and circumpolar flora of ancient, probably Tertiary distribution. As it returned to its original home in post-Pleistocene time this flora left remnants on high mountains in the south, but it also took with it representatives of alpine floras from these southern mountains, thus giving rise to the dual nature of the present array of arctic plants. Holm also pointed out the meridional subdivision of the southern elements: American ones in the American arctic, Asiatic ones in Siberia, *etc.* He made no attempt, as did Simmons, to define routes or sequence in the migration northward.

Agreeing in the main with Nathorst ('92), Holm considered the present arctic-alpine flora of the world to have evolved during the Tertiary on the great mountain masses formed at the beginning of that period: The European Alps, the Altai, the Rocky Mountains, and possibly also the Caucasus and Scandinavia. The existence of endemic genera in the modern arctic flora, however, and of distinctively arctic species in many other genera, led him to believe that a certain element had probably also developed in the far northern lowlands during the Tertiary. He also toyed with the idea of a certain amount of bicentric origin among closely related species.

Other writers of this period who still retained the Darwin-Hooker concept of large-scale migrations during and after the Pleistocene were Harshberger ('11) and Macoun and Malte ('17). All of these were concerned with the origins of all of the boreal flora rather than with the arctic alone.

The Nunatak Hypothesis. Thus far the premises upon which rested most of the thought concerning the origin and distribution of boreal plants were comparatively simple. Darwin, following ideas originally suggested by Edward Forbes ('46), had emphasized the idea of the *persistence* of plants in suitable localities after their former continuous ranges had been broken. The recognition of effective barriers to migration, as well as the failure to account for the means of migration in the cases of many species, had led to the

construction of a theory that would allow more time for the spread of plants. This was in contradistinction to the theory of multiple centers of species origin, and also to the theory of free and comparatively rapid contemporary distribution of plants over the whole surface of the land and their local sorting into communities by specific preferences.[2]

The extent to which the theory of persistence was to be carried, however, was not defined. It was used by Hooker in the case of arctic and alpine plants separated by great horizontal distances, and by differences in altitude which had set up strong ecological barriers. Here the uncertainty of the means of migration was sufficiently great to render the persistence theory easily the most attractive. If the theory were to be applied to smaller and more uniform areas, in which distance and other natural barriers were to become progressively less formidable, then more argument was due over the effectiveness of the means of migration.

Among the outstanding results of continued exploration in boreal America, and of increasingly critical study of the flora, has been the discovery of a great number of disrupted plant ranges and the "spotty" distribution, even in comparatively small geographic areas, of isolated species and endemics. There are far more of these than were known to Hooker, and they involve both arctic and temperate species. Arctic plants had been found not only on the mountains of New England and the western cordillera, but also at lower elevations in several districts about the Gulf of St. Lawrence where they were isolated both from their northern relatives and from each other. Furthermore, they exhibited a considerably higher percentage of endemism than their northern relatives. There was also the discontinuous distribution of specific segregation and endemism throughout all of the glaciated parts of the continent to be accounted

[2] An excellent contemporary statement of these ideas on geographic distribution is to be found in Sir Joseph Dalton Hooker's classic essay on the subject, delivered before the British Association in 1881: "Before the publication of the doctrine of the origin of species by variation and natural selection, all reasoning on their distribution was in subordination to the idea that these were permanent and special creations; just as, before it was shown that species were often older than the islands and mountains they inhabited, naturalists had to make their thories accord with the idea that all migration took place under existing conditions of land and sea. . . . Now under the theory of modification of species after migration and isolation, their representation in distant localities is only a question of time and changed physical conditions."

Hooker's essay also contains a valuable outline of earlier, pre-Darwinian thought on phytogeographic concepts.

for. Could the theory of persistence be utilized to explain the distribution of plants in such a small area as New England, for instance, or the region immediately surrounding the Gulf of St. Lawrence, without its being reduced to an absurdity in the face of the ordinary migratory abilities of the species?

Professor M. L. Fernald, in his "Persistence of Plants in Unglaciated Areas of Boreal America," attempted to apply the theory of persistence not only on a broader and more critical scale in all of boreal America than had been tried heretofore, but also in the complicated flora of New England, the Maritime Provinces of Canada, and Newfoundland. In doing this he raised new and perplexing questions, for the isolated and "persisting" colonies of plants were so close together or so unopposed by natural barriers that no ready explanations for their failure to occupy intervening territory were at hand except the old ones of bicentric origin and species preference. The problems of the boreal American flora were further complicated by the fact, first emphasized and elaborated by Professor Fernald, that the Gulf of St. Lawrence region contained a large number of species either identical with or very closely related to western cordilleran species. They were entirely isolated in the east, or had an intervening station or two in the upper Great Lakes country. Could these ranges also be explained by the theory of persistence, and why were the species not found in the intervening territory?

Professor Fernald placed the dispersal of relic arctic and cordilleran species, the results of which we now see, before the last ice advance of the Pleistocene. He conceived it most probable that the arctic-alpine plants persisted on ice-free lands in the arctic and upon small unglaciated areas (nunataks) well within or near the margins of the great ice fields. The cordilleran species in the Gulf of St. Lawrence region got their dispersal at least as far back as the last great interglacial, when conditions were suitable for their migration across the northern part of the continent, and persisted through the last glacial episode on ice-free areas along with the arctic plants. Still other plants using these nunataks as refugia in the same interval were species of distinctly southern dispersal on the Atlantic Coastal Plain. Their retreat southward had been cut off by the advance of the continental glacier to the southern New England coast, and later by the submergence of the continental shelf which had been their route of migration.

The so-called "nunatak theory," as outlined above, has had considerable support from glacial geologists who have admitted the existence of some areas about the Gulf of St. Lawrence, in the upper Great Lakes region, and possibly in the Torngat Mountain country of Labrador, which were not entirely engulfed by continuous ice-sheets. There is, however, as will be noted below, a great deal of argument on this score; and the undisturbed character of some of the areas is distinctly open to question, if not entirely disproved.

Conservatism vs. Aggressiveness. The behavior of many of the persisting relics has given rise to crucial problems. Why have they remained so local in their distribution, failing to utilize their means of spreading into adjacent country? To deal with this question Professor Fernald established the new premise that these species, being old, have become "conservative" or "non-aggressive," able to hang on to territory already occupied but limited in their ability to pioneer.

Here was a new departure in the reasoning of geographic botany. The first question asked by the plant geographer, after learning the identity of a species, is what determines its ability to grow where it does. Modern physiological plant geography emphasizes the interplay of factors in the external environment as of first importance in such matters. But if we must first condition the whole system of interrelationship by placing the species in a scale of inherited "conservatism" or "aggressiveness," neither of which is as yet a measurable quantity, then the whole problem becomes vastly more complicated. If it can be proved that such things as "conservatism" in species actually exist, and are effective in determining distribution, then the theory of persistence can be far more widely applied than it has been heretofore.

Professor Fernald's thesis was supported by an impressive body of critically examined fact, by far the largest that had ever been assembled in the study of boreal American plant distribution. There is no occasion to review it here, but various phases of it will appear in other parts of this paper. It should be noted that he still adhered to the outline of glacial sequence laid down by Tyrrell, and believed that the arctic flora acquired its modern relationship with alpine elements first in the west and later in eastern America. Furthermore, he considered the most ancient group of arctic-alpine plants to have had their center of dispersal in northwestern America

and northern Asia. One of the most cogent reasons for this is the large amount of endemism among the isolated eastern representatives of this group.

The most extensive support of the nunatak hypothesis as stated by Fernald has probably come from the botanists of the University of Montreal, chiefly under the leadership of Fr. Marie-Victorin. A large amount of collecting and critical study by these men along the lower St. Lawrence and in the Maritime Provinces has rendered even more formidable the mass of data with which any one attacking the idea must deal. At the same time various flaws in the argument have come to light, and the whole structure has been well summarized in a recent paper by Fr. Victorin ('38) which will be discussed further below.

One of the supposed refugia for plants during the last episode of the Pleistocene is in the Torngat Mountain region of Labrador. Several plant collections had been made there prior to the time when the nunatak hypothesis was formulated, and constituted the botanical basis for the suspected lack of glaciation. Still other collections have been made since, most of them from the lower elevations. All the records have been studied by Dr. Ernst C. Abbe who was botanist to the Grenfell-Forbes Labrador Expedition of 1931, and a searching analysis of the phytogeographic problems was published by him in 1936. The persistence of plants on nunataks in the Torngats was rendered extremely doubtful by the insistence of Odell, the geologist to the Expedition, that such nunataks did not really exist, and that even on the highest summits there is evidence of coverage by continental ice (Odell, '33). [See also Coleman ('21) and Daly ('02) for discussion of this area.]

The evidence for persistence in the Torngat region has rested upon 13 species of plants, five of which are of the western cordilleran region with a few isolated localities mostly in northeastern America, and eight are local endemics. According to Abbe's observations, all of these occur only at lower elevations near the sea coast, while the summits and higher slopes of the mountains have a sparse vegetation of hardy, wide-spread arctic plants. He argues from this that if the 13 critical species persisted at all it must have been at low elevations locally protected from some of the rigors of a glacial climate, possibly at the heads of fjords. Gelting has postulated an arrangement such as this for the persistence of relic plants in Green-

land ('34). Here again the recent geological interpretation of Odell is at variance, since he believes that even the coastal strip was not ice-free. Abbe advances another hypothesis: "namely, that during the postglacial climatic optimum there was an opportunity for the less hardy cordilleran species to move northward from refuges to the south" ('36). He points out that Dr. M. P. Porsild had already proposed the application of such a theory to problems in the American arctic in connection with his attempts to account for the southern elements in the flora of Greenland (3/5 of the total) ('22). On the other hand, Abbe also makes the significant observation that such a post-glacial migration of relic species "does violence to the concept of relics as senescent and non-aggressive species which have largely lost their power of migration." He cites, as support for a post-glacial migration theory, the findings of Fernald in the upper Great Lakes region ('35) and those of Nordhagen on the Atlantic coast of Norway ('35). Fernald proposed that several plants now rather widely distributed in the Upper Great Lakes region survived Wisconsin Glaciation on the Keweenaw Peninsula. Nordhagen suggests that relic plants which persisted on ice-free lowlands of the Norwegian coast were forced by competition to migrate, in post-glacial time, inland to calcareous mountains which are known to have been uninhabitable during the glaciation.

With regard to the eight species which are Torngat endemics, commonly used as evidence of age in the flora, Abbe proposes that the evidence is not of primary importance because it occurs in such groups of plants as *Antennaria* and *Taraxacum* which tend to produce seed without fertilization. By this means even minor mutations are apt to be perpetuated rather than submerged by backcrossing. Hence, they are likely to be of less value as indicators of long lapses of time than if free interbreeding occurred.

Pennell ('34), in dealing with the distribution of the species of *Castilleja* in Alaska and northwestern Canada, has related their development and survival to the distribution of glaciated and unglaciated parts of Alaska. The local endemics are mostly associated geographically with the unglaciated Yukon Valley, while certain other wide-ranging "aggressive" species have occupied the territory formerly covered by the last glaciers, spreading far eastward to Hudson Bay.

Wynne-Edwards' Criticism of the Nunatak Hypothesis. The most comprehensive attack yet leveled at the nunatak hypothesis is

probably that of Dr. V. C. Wynne-Edwards of McGill University who, in connection with ornithological field work, has travelled widely in the eastern Arctic, the region about the Gulf of St. Lawrence, and in northern New England ('37, '39). The main objections enumerated are five in number, as follows: First, there is serious doubt that the relic species could have withstood the rigorous glacial climate of such ice-free areas as existed. This is particularly true of the cordilleran plants among them, many of which now require relatively sheltered habitats.

Second, some of the relic localities such as those at Bic, Rimouski Co., Quebec, and on the Mingan Islands and Anticosti, either contain no actual nunatak areas, have exceedingly small ones, or were covered by the post-Pleistocene invasion of the Champlain Sea. At Bic it is possible that a very small area stood above the ice, but it could hardly have served as refuge for some of the less hardy western species now localized in the vicinity. Abbe's theory of persistence at lower levels along the coast is untenable because the ice covered the sea.

Third, Wynne-Edwards maintains that the behavior and distribution of cordilleran and arctic elements in the flora of the east indicate that they can not be subdivided in any analysis of their dispersal. It should be remembered that Fernald drew a distinction between ancient arctic-alpine types of western America and northern Asia, on the one hand, and youthful arctic species, on the other. The former, according to Fernald, are of restricted range in the east, and constitute the relic species, with a high percentage of endemism among them; the latter are widely distributed in arctic and alpine situations and have produced very little endemism.

Fourth, using tentatively as premise the idea that large endemism is indicative of great age, Wynne-Edwards suggests that if both cordilleran and circumpolar arctic floras in America are older than the Wisconsin glaciation, as they are commonly regarded to be, then differing amounts of endemism between them can have no significance as a criterion for subdividing them as relic or non-relic groups on supposed nunataks. He also suggests that a survey of endemism in nearctic and holarctic types in the Arctic flora of northeastern America would not yield great differences.

Fifth, it is maintained that the "spotty," localized occurrence of isolated or endemic species can be correlated best with soils, par-

ticularly those rich in lime or in combinations of magnesium, lime and soda; and that the presence or absence of Wisconsin glaciation is of no consequence.

In place of the nunatak hypothesis, Wynne-Edwards proposes that we return to the classic concept of Hooker: "that the arctic-alpine flora of eastern Northern America has formed a single unit since pre-Wisconsin times; that in those times it occupied suitable habitats in latitudes similar to or higher than now; that with the advance of the Wisconsin glaciation it was driven southwards and outwards; and finally with the retreat of the ice a recolonization of suitable habitats took place." To account for locally disrupted ranges he relies upon the soil preferences of plants and their ability to disperse themselves freely over considerable intervals of uninhabitable territory. He accounts for the lack of uniformity even in the favored lime habitats by reverting to the time factor. As for the prevalence of endemism among certain elements of the flora, he relies upon our scanty knowledge of the actual causes of this phenomenon, indicating that it may be correlated with isolation as well as time, and noting, as does Abbe, that many of the species complexes are of a "plastic" nature, throwing off permanent segregates more or less easily.

Marie-Victorin ('38) has christened this return to earlier concepts, with slight modifications of his own, the "rainbow explanation" since it envisages a sort of arch, one of whose limbs is in the western cordillera and the other in the Gulf of St. Lawrence region, with the central portion in the arctic. In pre-Wisconsin time it was the highway for free dispersal among cordilleran and arctic species, but now contains the troublesome broken ranges of eastern, western and arctic types. As would be expected, the validity of the whole structure depends rather heavily upon some sort of barrier preventing the free interchange of species across the center of the circle. Since no topographic or climatic ones are available, except the partial division by Hudson Bay, Wynne-Edwards rests the case upon the failure of the "soil-specialized" species to occupy the great expanse of acidic rocks in the Canadian Shield.

Discussion of Wynne-Edward's Criticism. Many other students have questioned the physical possibility of the survival of plants on nunataks (Abbe, Fernald, Raup, Marie-Victorin). It is a matter decidedly open to question, but as Abbe ('36) has noted, "it is im-

portant to remember . . . that the solution applicable in one area is not necessarily applicable in another area different in physiography, latitude, and its geological conditions." Thus the "St. Lawrence region was far better situated during the Wisconsin for the survival of plants at higher elevations (as well as at lower levels), because of the large areas left uncovered by ice, a warmer climate associated with its more southern latitude, and the predominance of basic rock. On the other hand, northern Labrador with its predominantly acid Archean formations, its more northern latitude and correspondingly more stringent climate, and greater (?) covering of ice was hardly as favorable for the persistence of flowering plants." The occurrence of vegetation near or on the margins of existing glaciers is often advanced as evidence that survival on nunataks was possible, but here also it is essential to keep local conditions in mind.

The presence of relics at such places as Bic and the Mingan-Anticosti area constitutes a problem similar to that in northern Labrador, should the findings of Odell stand confirmed. Marie-Victorin ('38), after an exhaustive examination of the Mingan Islands and Anticosti, still clings to the idea of the persistence of relics there, shielded during the Wisconsin glaciation between tongues of the glacier, and spared during the invasion of the Champlain Sea by a smaller submergence than has been thought.

Marie-Victorin considers that the weakest point in the nunatak hypothesis is probably the subdivision, by Fernald, of the arctic-alpine flora into cordilleran and arctic types. He is inclined to follow Wynne-Edwards in considering the two elements as inseparable, but containing plants, some of which "have wide limits of climatic tolerance, . . . while others are more narrowly confined . . ." to the lower portions of the "arch" noted above. In this connection he raises the significant question of negative evidence, upon which all disruptions of range must, in last analysis, rest. He suggests that the "rainbow explanation" might be more adjustable to the possible filling of gaps than the nunatak hypothesis.

Some of the writer's findings in the arctic and alpine flora of the Peace River country may have some bearing upon the similarity or dissimilarity of behavior between the two ('34, pp. 61–3). The Peace River flows eastward through a pass in the main range of the Rockies at an elevation of about 2000 feet. The nearby peaks tower

to 7000–7500 feet, with timber line between 3500 and 4500 feet. Forty species of vascular plants found above timberline were also found on the river banks or upon rock slides very near them. Most of these inhabit damp sandy or muddy banks close to the water's edge where they are inundated in flood times, making their situation exceedingly unstable, and the existence of any plants at all quite hazardous.

The striking feature of this group of forty species is the preponderance of forms with widely extended ranges in arctic or north temperate regions. Only six of them are distinctly cordilleran, and these are all derived from the lower part of the alpine zone on the adjacent mountains. On the other hand, one wide-ranging species found on the summit, and 19 of those from the 5000–6000 foot zone are present. It appears, therefore, that the greater part of the alpine cordilleran species (and nearly all of the high alpine ones) remain strictly alpine in this latitude, whereas species of wider range in arctic and subarctic regions are less selective and are to be found at the highest and lowest elevations within the mountains. This difference in behavior may be analyzed in at least two ways. It may be considered that by ordinary means of dispersal all available habitats in this region of subarctic conditions which are suitable for alpine species would be occupied by them; and the river banks and low rock slides are the only places at or near river level which are available. If this is the case, we must suppose that the cordilleran alpine species are nearly all so different in structure and function from the arctic ones that they are unable to effect this short expansion of range, although the two groups exist side by side at higher elevations. Again, we may look upon the northern cordilleran region as a vast area of environmental overlap in arctic and northern forest conditions. To be consistent with what we know of the postglacial history of these northwestern floras, we must consider the forest as having invaded a vegetation resembling the modern arctic tundra. The mountain sides were covered to such elevations as the local montane climates would permit, the tundra persisting only at the higher elevations. At the same time there could have been an invasion of this relic tundra by cordilleran alpine types. The existence of arctic-alpine plants at low levels suggests that some elements of the tundra persisted also in such lowland habitats as the forest was unable to invade because of the general instability of the soil.

In whichever way the matter is viewed, there seems to be a distinct difference in behavior between cordilleran and widespread arctic elements in the Peace River region.

Both Marie-Victorin ('38, p. 548) and Fernald ('33, pp. 120–7) have noted the common occurrence of cordilleran relics or elements of the arctic flora at or near sea-level in the Gulf of St. Lawrence region. Fernald has described the situation for the coast of western Newfoundland in some detail, and has interpreted it as a function of local habitat selection among the relic species: "It would seem, then, that the persistence of so extensive an element of the Arctic flora along the outer coast of western Newfoundland is due more to the aridity and the calcareous nature of the exposed ledges and the deep mantle of weathered debris than to low temperature; and that the flora of the rock barrens of western Newfoundland is largely composed of Arctic species because on these barrens are found arid conditions and calcareous soils comparable with those of much of the Arctic Archipelago. . . ."

Marie-Victorin has advanced another hypothesis for such anomalies as he found in Gaspé, Anticosti and Mingania. He thinks that some of these cordilleran plants at sea-level may have migrated there in post-glacial time by way of the shores of the Champlain Sea. It has been shown by Potter that there are a number of sea-shore plants on the coast of James Bay which are entirely isolated from their kind in the Gulf of St. Lawrence ('32). Potter has proposed that they arrived there by way of the shores of a hypothetical sea connection during the Champlain subsidence, and has adduced a considerable body of evidence that such a sea route actually did exist. Marie-Victorin also suggests that some of the cordilleran relics might have migrated eastward in the "dry unforested belt that must have existed along a receding ice-front, as a kind of side-walk extending from the Rockies to the Gulf of St. Lawrence . . ." ('38).

So far as the writer is aware, no one has attempted to apply to many of these eastern floristic problems the probable effects of mass movements in vegetation. The whole is greatly complicated, of course, by the likelihood of a post-glacial climatic optimum which must be taken into consideration.

Returning to Wynne-Edwards' fourth objection to the nunatak hypothesis, he is probably correct in questioning the significance of

endemism as a measure of time alone. But whether we regard it as a function of time, isolation, or the genetic character of the species, it is difficult to lay aside the coincidence of geographic differences in the amount of endemism. It is striking that after several years of collecting and critical study in the eastern arctic flora, Dr. Polunin could describe only four well defined new species; whereas nearly every collector who goes to northern British Columbia, Yukon or Alaska returns with something entirely new to botanical science and apparently limited to those countries. The concentration of endemism in the Gulf of St. Lawrence region and Labrador has already been noted.

With regard to Wynne-Edwards' insistence upon the geographic significance of soil-specialization, Marie-Victorian points out that in spite of the fact that many plants do show more or less exacting soil preferences, ". . . one cannot without danger reverse the statement, nor can one use safely the chemical explanation of distribution, except as applied to very general situations. Without reopening a discussion that was the great ecological theme in pre-Warming days, it seems certain that some plants may be chemically indifferent under certain conditions of latitude or climate, and calcicolous being given another set of conditions. For instance, *Acer nigrum, Ulmus racemosa, Celtis occidentalis,* not distinctly calcicolous within their main areas, are almost exclusively so in southeastern Quebec where they reach their northern limit. And it so happens that many plants of rather indifferent preference can maintain themselves in the north only by creeping on the limestones of Mingania, Anticosti and similar regions" ('38, p. 546). Simmons' observations in the arctic archipelago on this score are also of interest ('13, p. 135): "There are, indeed, not few species found on rocks of a certain system alone, but in no case there is any necessity for assuming the kind of rock to be responsible for this circumstance, other explanations always being more satisfactory. We may, therefore, I think, assume that every species in the flora can grow on any kind of rockbottom as far as the chemical nature of the soil is concerned, but some species more or less conspicuously prefer certain rocks, or at least avoid some."

If we return to the old Hooker concept, as suggested by Wynne-Edwards, then certain corollaries of it must also be revived. We are again faced with the question of free dispersal. Can any species

be assumed to be potentially able to occupy any piece of ground, provided the latter falls within a rather wide range of climatic tolerance, and within the range of the species' soil specialization? Wynne-Edwards appears to think that it can, giving to vascular plants in general "more or less efficient methods of dispersal . . . they are sometimes able to make strides of 500 kilometers at a time . . . attested by the colonization of oceanic islands" ('37, p. 20). Professor Fernald, impressed by the prevalence of discontinuity among plant ranges, has denied to certain species this ability for ready movement and establishment; and has called them conservative, or senescent, in contrast to a great body of aggressive species able to occupy more territory.

Since he returns to the concept of greater freedom of migration, Wynne-Edwards must account for the absence of many of the eastern relics in the vast Canadian Shield by the absence there of the basic rocks to which they are specialized. One of the outstanding features of the geology of the Canadian Shield is the prevalence of acidic rocks: granites, gneisses and schists; but it is also noted for widely scattered mineralized areas invariably associated with pre-Cambrian sediments which are highly metamorphosed and deformed.

Owing to the presence of economically valuable minerals in rocks of this kind, the Shield has been, during recent years, the scene of considerable survey activity and prospecting. The results of this were summarized in 1932 for the Northwest Territories by C. H. Stockwell and D. F. Kidd of the Canadian Geological Survey as follows ('32):

"The eastern area is an immense quadrangle extending from the north boundaries of Saskatchewan and Manitoba to the Arctic, and from the basins of Great Slave and Great Bear lakes east to Hudson Bay. . . . It has been geologically explored only along a few main routes, but the information thus obtained makes plain that the area is occupied by Precambrian rocks and that, broadly speaking, the main geological features are like those of other, better known parts of the Canadian Shield. The oldest rocks are assemblages of sediments and volcanics. The areas of these rocks are cut and surrounded by granitic and gneissic rocks which occupy by far the greater part of the region. Younger than most of the granitic rocks are local developments of sedimentary rocks, some much younger than others.

"The ancient assemblages of sediments and volcanics vary in character from district to district, but as a rule are much altered. In general, they resemble the schistose rocks which in the southern part of the Canadian Shield form the Keewatin volcanics and the associated ancient sedimentary groups and in which most of the valuable mineral deposits there known have been found. . . . Areas of these rocks are known to exist: (1) southwest of Rankin inlet along the Hudson Bay shore and inland to the southwest for a great unknown distance; (2) northwest of Baker lake which lies west of Chesterfield inlet, Hudson Bay; (3) at two places on Kazan river which enters Baker lake from the southwest; (4) on parts of the north and south shores and on some of the islands of Great Slave Lake; (5) on the east shore of Great Bear Lake; (6) near Point and Redrock lakes on Coppermine river; and possibly (7) west of Bathhurst inlet. . . .

"The youngest groups of strata, those that so far as known are younger than any of the granitic bodies, are extensively developed in several districts. In the eastern part of Great Slave Lake they consist of at least two groups of sediments and volcanics relatively unaltered though penetrated by dykes and sills. Large areas of relatively undisturbed sediments are found on the shores of Baker, Schultz, and Aberdeen lakes with long tongues extending southwest to Dubawnt lake and west for a long distance up the valley of the Thelon river" (pp. 70–71).

South of the 60th parallel, in the vicinity of Lake Athabaska, similar sedimentary rocks have been described by Camsell ('16*a*) and Alcock ('36).

Dolomites or limestones have been described in both the older and younger series in the eastern part of Great Slave Lake, and in the older group in the country north and northeast of Lake Athabaska. They have also been noted in the area southwest of Rankin Inlet. It seems not impossible that small outcrops of rocks of this sort will be found elsewhere as exploration goes forward. The present writer has collected in a number of localities around Athabaska Lake, and on the east and north arms of Great Slave Lake, with some results that may have some significance here. The dolomitic rocks have a rather distinctive topography and vegetation among the surrounding, harder materials. They have weathered to form soils which, though thin, are much better than have been

produced on the granites and greenstones; and hills made of them have more rounded summits than those of harder rocks.

These dolomites harbor some plants which are pronounced calciphiles and have not yet been found elsewhere in the pre-Cambrian parts of this district.[3] They represent both arctic and subarctic or north temperate elements of the flora. The whole vascular flora of the pre-Cambrian part of the district, as now known, contains 423 species. About 25.8% of them (109 spp.) are also to be found in the flora of the eastern Arctic, as listed by Polunin (making up about 36.7% of that flora); approximately 2.2% (9 spp.) are endemic so far as they are yet known; and the remainder, 72%, are plants of wide range in subarctic and north temperate America. A few (probably less than 10) are distinctly western in affinity, here reaching their eastern limits, while another small portion here reaches western limits.

If Wynne-Edwards' thesis is correct, the calcareous hills in the Athabaska-Great Slave Lake district should be inhabited by a considerable number of the cordilleran plants that are isolated in the St. Lawrence region. General climatic conditions, as indicated by the whole flora, are sufficiently moderate to allow the growth of many of the less tolerant cordilleran species. It is of interest that several of these plants do occur there, some of them, like *Woodsia scopulina, Arenaria cylindrocarpa* (=*A. humifusa*), *Woodsia oregana* and *Arenaria macrophylla,* constituting midway and rather high northern stations for the most pronounced of the epibiotics. However, Fernald listed 225 western and endemic species centering about the Gulf of St. Lawrence, only 35 of which, or about 15.5% have been found in the pre-Cambrian districts noted above.[4] This

[3] *Woodsia oregana, Woodsia glabella, Ribes lacustre, Lychnis Drummondii, Pellaea glabella, Carex scirpoidea, Carex glacialis, Carex eburnea, Taraxacum ceratophorum, Arenaria dawsonensis, Arenaria humifusa,* etc.

[4] *Woodsia oregana*
Woodsia scopulina
Juniperus horizontalis
Calamagrostis canadensis var. *robusta*
Carex concinna
Carex Garberi
Juncus Vaseyi
Salix myrtillifolia
Betula microphylla
Geocaulon lividum
Arenaria dawsonensis
Arenaria humifusa
Arabis divaricarpa
Potentilla pectinata
Rubus acaulis
Dryas Drummondii
Astragalus eucosmus
Astragalus frigidus var. *americanus*
Hedysarum Mackenzii
Hedysarum alpinum var. *americanum*
Arctostaphylos Uva-ursi var. *adenotricha*
Dracocephalum parviflorum
Rhinanthus Kyrollae
Solidago multiradiata

seems rather small representation if, as Wynne-Edwards suggests, the only reason the disrupted ranges have not been filled in is the absence of calcareous soils. Another significant fact about the Athabaska-Great Slave Lake flora is that its known endemics are not found on the calcareous rocks, but on sands derived from pre-Cambrian sandstones south of Lake Athabaska.

Thus the evidence from the isolated pre-Cambrian dolomites is not conclusive; but rather, tantalizing. If some of the most notoriously discontinuous ranges, thought to be due to the invasion of the Pleistocene ice, can be found to have intermediate stations deep in the most heavily glaciated territory, might not others be found also? But in spite of rather promising conditions a mere handful have been turned up; and there are still the problems of speciation and endemism mentioned previously.

Griggs, in a recent paper ('40) on the occurrence and behavior of rare plants, suggests that the isolation of many species is due not so much to historical causes as to their failure to compete successfully. He notes that many such species grow in situations of unstable habitat or the initial stages of successions where the dominance of other plants is not so well defined. This proposal might be called a phytosociological substitute for Wynne-Edwards' soil-specialization theory. Wynne-Edwards himself suggested such a substitute in his second paper on the subject ('39). It may account for much of the local distribution of the isolated species, but does not clarify their means of arriving in their present areas or the reasons for their not having spread to larger areas.

One of the most striking and complex phases of the nunatak hypothesis relates to the coastal plain flora of eastern America. This flora is well represented in parts of New England, the Maritime Provinces and Newfoundland; and Fernald ('11) has shown that 35% of the whole vascular flora of Newfoundland is of southern affinity, common either to the New England-Acadian coast or to the coast south of Cape Cod. If we return to the idea of easy dispersal, even in long "jumps" over land and sea barriers, then there is no problem in accounting for the presence of these southern

---

Arenaria macrophylla	*Antemisia borealis*
Cerastium Beeringianum	*Petasites vitifolius*
Ranunculus Purshii	*Arnica chionopappa*
Anemone multifida var. *hudsoniana*	*Senecio indecorus*
Anemone parviflora	*Senecio pauperculus*
	Taraxacum lacerum

plants in Newfoundland. But if we recognize limitations in the ability of plants to migrate, then the problem of northern, isolated representatives of the coastal plain flora becomes acute, as it does with other discontinuous ranges.

The current explanation for the northern isolation of coastal plain plants is based upon an ancient emergence of the continental shelf from the sea, Fernald ('11, '33, '40), Nichols ('13, pp. 98–9), Barrell ('15), Martin ('25). A similar hypothesis has recently been applied to the distribution of land snails by Brooks ('36). This would give the necessary pathway along which plants could migrate as far as Newfoundland. The geological history of the shore line, as now interpreted (Johnson, '25; Fernald '33), pushes the time of this dispersal back to the late Tertiary or early Pleistocene, and we are confronted with the problem of getting hundreds of southern plants through the rigors of the glaciation on refugia in Newfoundland and the Maritime Provinces or on the nearby exposed continental shelf. As previously noted, arguments still rage over the existence of ice-free surfaces in Newfoundland and parts of the Maritime Provinces during at least the later parts of the Pleistocene, in a region otherwise covered by continental ice; and if they did exist, whether the southern plants could have withstood the peri-glacial climate of the time.

The theory of a post-glacial optimum during which the southern plants might more easily have migrated northward could be applied here as well as in Labrador where it was suggested by Porsild and Abbe. It is subject to the same limitations as in Labrador, however, if we question the ability of the plants to disperse themselves in the comparatively short time since the Pleistocene or since the advent of the post-glacial warm period. In any case the nunatak hypothesis can hardly be successfully demolished unless the coastal plain element is satisfactorily disposed of on some other basis.

Marie-Victorin sums up the arguments, pro and con, over the nunatak hypothesis as follows: ". . . the situation seems much too complex for one good simple, schematic and dogmatic explanation. There is evidently a general truth in Hooker's hypothesis of a back and forth 'mouvement d'ensemble' of the whole flora of eastern Canada during and after Pleistocene times. There is also much in favor of the nunatak theory, which may be necessary or helpful to explain a number of cases. How else can the presence of *Agoseris*

gaspensis on Table-top Mountain, of *Salix brachycarpa* and *Polystichium mohrioides* on Mt. Albert be explained, nothwithstanding the magnesian preference of the latter species?" He also raises the question as to whether one of the major premises involved is justified: "Is the conception of a senescent preglacial or interglacial flora, vanishing gradually from northeastern America, defensible on biological grounds? Are there really such things as senescent species, and senescent floras in the present world?" ('38, pp. 553–4).

Thus we are brought back to the questions of time and distance in plant dispersal, and of the ultimate capacities of species to disperse themselves. As noted previously, whether we can apply the theory of persistence in detail to comparatively small parts of the earth's surface as well as to large ones depends very much upon whether there *is* a classification of plants on the basis of their capacity to travel and adapt themselves, and if so, whether we can find a key to the system. This brings us to the consideration of one of the most recent and possibly the most significant analysis of the boreal biota—that of Dr. Eric Hultén.

Hultén's Studies of Arctic and Boreal Biota

Statement of the Problem. Dr. Eric Hultén, after exhaustive floristic studies in Kamtchatka and the Aleutian Islands, has drawn up an "Outline of the History of Arctic and Boreal Biota during the Quarternary Period" ('37*b*). He has mapped the ranges of hundreds of boreal species, organizing his factual material on the basis of what are called "equiformal areas." That is, when large numbers of ranges are superposed they fall into a group of patterns which are more or less clearly defined geographically, and "equiformal" within themselves. Each equiformal area shows a region of concentration in number of species, which is called its "centre." Since it is assumed that each species has acquired its present range by dispersal from a point of origin, the regions of concentration within the equiformal areas are regarded as fundamental centers of origin for the various major elements in the flora; and the "equiformal areas" become "equiformal progressive areas" for the origin and development of the flora. The species are termed "radiants" from the different centra. Those species which are more or less confined to the centra are termed "centrants."

The geographic arrangement of centra as worked out by Hultén is as follows. They are ". . . in North-Eastern Siberia and in the

Amur–Manchuria region. Another occurs in the Altai–Sajan region, sending out radiants towards the Arctic shore. A third centre is northern Japan, whence numerous plants radiate to the north and to the coast of the Asiatic Continent. A centre of great importance is the region around the northern part of the Bering Sea. It sends out progressive radiants reaching symmetrically as well to the west into arctic Asia and Europe as to the east to Eastern America, and also often extends arms along both the Asiatic and American Pacific coast. In America radiants proceed from the Yukon valley along the Arctic American coast, others centre around the Arctic Archipelago, and others again have the centre of their progressive figures in the State of Washington and radiate along the American coast or along the Rocky Mts. to Alaska. Of the plants discussed in this paper no groups could be formed having their centres in northern Europe or western Siberia, or in North-Eastern America or in the country between Yukon Valley and the Great Lakes" (p. 25).

The author immediately draws a correlation between the distribution of his centra and the distribution of ice during the Pleistocene. The areas in which he could locate no centra are those "districts which were covered with ice during the maximum Pleistocene glaciation. All plants of our area radiate from districts that were not completely buried under the ice-sheet of the maximum glaciation. In other words: the plants have spread over the arctic and boreal belt from the refugia close to the ice, where they were left in positions of a small part of their earlier area, and where they were able to survive the severe conditions of maximum glaciation" (p. 25). Since the history of the Pleistocene looms so large in Hultén's explanations of boreal distribution, he devotes considerable space to a review of the most recent findings concerning it (see pp. 19–20).

The relic hypothesis for the explanation of plant distribution is applied by him not only on a broad continental scale as noted above, but also in dealing with comparatively small areas. A good illustration of this is to be found in the discussion of radiants from southern Beringia. After a detailed study of the flora of the Aleutian Islands and nearby territory the following conclusions concerning its distribution were drawn up. It was found that a considerable number of species had disrupted ranges in a long strip of coastal territory around the north Pacific, chiefly on the northwest continental coast, the Alaska peninsula and the Aleutians, the Commander Islands,

southwestern Kamtchatka, the Kuriles, and as far to the southwest as Saghalin. It is concluded that these disruptions can hardly be incidental as "proved by the fact that if we consider all plants occurring within the region of the group discussed, the gaps are found to occur again and again in the same places in the areas of the most different plants. The phenomenon could be described as follows: the areas of all species of the group discussed are composed of smaller elements which are united to a complex. The gaps between the elements might be distinguishable, but in other cases they might be undiscernible. In different species the gaps always occur in approximately the same places. Another important circumstance is that there occur endemic plants which exactly or approximately occupy the area of such an element or of two or more elements combined" (pp. 40–41).

By way of explanation there is the assumption "that the plants in question already occupied at least the greater part of their present area earlier than the maximum glaciation and that this old area was split up into parts separated from one another by heavy glaciation or by districts with such severe climatic conditions during maximum glaciation that the species concerned were exterminated there. After the maximum glaciation, when the amelioration of the climate set in, the isolated partial areas, which I propose to call elementary areas, started to send out progressive radiants in all available directions and in this way the old original areas of the plants were more or less completely restored" (p. 42). Hultén then proceeds to divide the whole district up into four "elementary areas," and shows that they can be so correlated with what is known of the glaciation and its attendant conditions.

Plastic vs. Rigid Species. It will be seen at once that if such an hypothesis is to be made workable, it must deal successfully with the same problems concerning the capacity for dispersal which confronted Professor Fernald in his analysis of the American boreal flora. In studying the southern Beringia radiants noted above, Hultén found that some of the species were able to occupy far more of their old ranges than others, and that it was necessary to draw up some sort of a distinction between them.

To do this he set up a premise explained in different words and approached in a different manner, but essentially like that by which Fernald distinguished between "conservative" and "aggressive" spe-

cies. This premise becomes a corner stone of Hultén's entire argument, and will bear further examination.

Hultén recognizes "plastic" and "rigid" species. The plastic ones are those which are able to migrate rather freely and adapt themselves to new homes; while rigid ones are those which for some reason have wholly or in part lost their ability to do these things. The idea of senescence, drawn largely from paleobotany, is not relied upon to explain this so much as the history of genetic character within the plants. "Within a given species there is always a certain potential variation. Under the influence of a catastrophe such as the glacial period, when large parts of the area of the species in question are covered with ice and the rest is severely exposed to unfavorable conditions, a reduction of this variability is inevitable, as all biotypes, being more sensitive to the hardships, are exterminated." And, "A very strong reduction of the biotypes is bound to ensue and, parallel to it, a strong reduction of the potential variability and thereby also presumably the spreading capacity" (p. 22). And again, discussing the southern Beringia radiants, "That the splitting up of the earlier areas by the ice . . . must have had a very important influence on the number and character of the biotypes remaining within the different elementary areas can hardly be denied. It is evident that the selection of biotypes within the northern elementary areas must be different from that within the southern. When the ice recedes and the plants start to migrate from the isolated spots where they were left, the populations in the different elementary areas must differ in their composition. At any rate those to the north must differ considerably from the original population which had occupied the same stations during the preceding interglacial. In other words: different races of the species must have been formed under the influence of the ice. As long as those races are separated from one another geographically they may possibly be distinguishable, but when the migration has proceeded so far that the radiants from two elementary areas meet, hybridization and thereby an integration of the differences must be expected to occur." "Different species naturally react very differently to the vicissitudes. Some keep their preglacial type unchanged or very little changed in the most remote districts of their area. This presumably applies particularly to the very old types which had already undergone vicissitudes in one direction or another and have thus already been de-

pauperated in more respects than one. What is left of their original set of biotypes is a population that has been able to withstand all the different conditions to which the plant has been subject during the geological ages. They are probably very poor in biotypes and have but a small potential variability. Other species change comparatively easily into new forms under a pressure such as that discussed above; they might be more recent types that have not yet been so depauperated as to lose a considerable part of their potential variability" (pp. 47–48). He suggests that "in some of the areas isolated by the ice the conditions became so severe that certain species could not survive and therefore now show a gap in their distribution there. In other cases the plants in such an isolated area were so depauperated of biotypes that they lost their spreading capacity and are still found only in the stations where they were left by the maximum glaciation. They were thus transformed into rigid species."

Hultén maintains that the "Linnaean species" of the boreal flora originated in the last great interglacial or earlier, and that "the present areas of arctic and boreal biota are on the whole reductions from the areas which had developed already during the great interglacial." Smaller segregations within the Linnaean complexes are thought to have arisen later. One of the conclusions of the whole study which he conceives to be of first importance is "that the vascular plants ordinarily spread much more slowly than has been commonly assumed. World-wide postglacial areas do not exist, except in the case of weeds introduced by Man."

An interesting corollary to the theory of plastic and rigid species as outlined by Hultén is the significance that may be attached to the *size* of the relic areas and relic communities. Very small refugia could contain only small relic populations of plants, and the reduction of biotype material would be much greater than in the case of large areas or the region south of the ice sheets, wherein larger populations could exist, with more biotypes and more opportunity for genetic interchange. This corollary is made use of throughout Hultén's analysis, with striking results.

Another subsidiary idea is that in general the centrants are apt to be plants belonging to polymorphic genera, and taxonomically not very highly differentiated. In contrast, those of wider range are likely to have been subjected to a variety of conditions, to have been

in existence for longer times, and to have been affected by isolation and the depauperation of biotypes. Their specific segregation could be expected to be more pronounced and more stable than that of the centrants. Even though they might be very old radiants, on the other hand, if they were able to maintain large populations in spite of such cataclysms as the glaciation they would still retain their vigor and spreading capacity.

Still another phase of the matter which is of first importance is that a given species may retain its plasticity in one area and lose it in another. Thus a cordilleran species might be widespread and aggressive in the Rocky Mountains where it is thought to have had ample space during the Pleistocene; while in the Gulf of St. Lawrence region it was confined to small refugia and so depauperated of biotype material as to have become rigid, or conservative.

One of the more far-reaching conclusions arrived at from these lines of argument is that the present distribution of species cannot be correlated directly with modern climates alone. It must be considered first in the light of the history of the plants themselves—their former ranges, the amount of discontinuity in their ranges, their degree of specific segregation, and their present potential variability. This idea is inherent in the nunatak hypothesis as elaborated by Fernald ('25), has been stated by Abbe with regard to his Labrador problems ('36, '38), and was noted by Livingston and Shreve after their exhaustive studies of vegetation-climate correlations in the United States ('21). Griggs has emphasized it in his analysis of Alaskan timber-line conditions ('34*b*, '37). It has recently been stated clearly by Faegri ('37*b*, p. 429) in connection with problems in Scandinavian phytogeography.

The idea of the genetical effects of isolation as outlined above was developed by the Dutch students of evolution, A. L. and A. C. Hagedoorn ('21), and has been elaborated in its relation to taxonomic problems by Du Rietz ('30). So far as the writer is aware, Hultén's is the first application of it to broad phytogeographic problems involving whole floras—certainly the first in America.

Dobzhansky ('37, pp. 133–4) has summarized much of the recent research on the size of populations, referring particularly to the work of Sewell Wright ('31, '32), as follows: "Wright considers the situation that may present itself in a species whose population is subdivided into numerous isolated colonies of different size, with the

exchange of individuals between colonies prevented by some natural barriers or other agents . . . such a situation is by no means imaginary; on the contrary, it is very frequently encountered in nature. In the course of time such a species will become differentiated into numerous local races which will differ from each other in the frequencies of various genes. The populations of the separate colonies at the time of their isolation may be assumed to be all alike in their genetic constitution. Some colonies, especially the large ones, will preserve the constitution of the ancestral population approximately unchanged. Each of the colonies with very small breeding populations will soon become genetically uniform owing to the depletion of the store of hereditary variability they once possessed. It is important to realize that in different colonies different genes will be lost and fixed, the loss or fixation being due . . . simply to chance. Hence, at least some of the colonies will become genetically distinct from others, giving rise to local races. Finally, populations with intermediate size will likewise become distinct from each other and from the ancestral population, but the supply of the hereditary variability present in them will not be depleted so drastically as in the very small ones. That is to say, the colonies of intermediate size will preserve a certain amount of the evolutionary plasticity which will be lost in the small ones. . . .

"The conclusion arrived at is an important one: the differentiation of a species into local or other races may take place without the action of natural selection. A subdivision of the species into isolated populations, plus time to allow a sufficient number of generations to elapse (the number of generations being a function of the population size), is all that is necessary for race formation. This statement is not to be construed to imply a denial of the importance of selection. It means only that racial differentiation need not necessarily or in every case be due to the effects of selection."

Studies by Turesson ('22) in the old world, and by Clausen, Keck, and Heisey in California ('40), have greatly clarified the genetic concepts of minor environmental modifications and of heritable racial differentiation. Some of the conclusions reached by Clausen, Keck, and Heisey are worthy of quotation in the present connection (pp. 408–9): "Variation, of course, is of two sorts: that which is controlled by environment (modifications), and that which is governed by the heredity (genetic differences). Both contribute

to the differences seen, not only between species and races, but also between individuals of one population. . . . Species with the widest distribution are observed to have the most races. Conversely, species of narrow distribution have fewer races and are less variable. . . . The regional races of a species are heritably distinct ecotypes, not modifications." These studies are based on a large series of transplant experiments in the coastal, mountain, and desert regions of California between 1922 and 1938.

Anderson ('36) has applied these ideas not only to the formation of races, but also to the differentiation of "conservatism" as understood by Fernald, using certain forms of *Iris,* particularly the relatives of *I. setosa.* In this group an Alaskan series proves to be highly polymorphic, while an eastern group, isolated around the Gulf of St. Lawrence, is remarkably uniform. "This conservatism of *Iris setosa* var. *canadensis* is distinctive of most of the glacial endemics (or near endemics) of the region around the Gulf of St. Lawrence. In one of his classic contributions to the subject, Fernald ('29) has aptly characterized them as 'already waning types, too old, or too conservative to spread into closely adjacent and virgin soils.' In the case of *Iris setosa* var. *canadensis* the invariability cannot be a direct effect of time, for the highly variable irises of Alaska are quite as aged. It is more probably, as Professor Fernald has suggested, an innate conservatism; a conservatism founded genetically upon the fact that these irises are descendants of a small and highly selected stock. Hard times removed from the region all the luxuriant types which may once have existed there. When the ice age was over the immediate area was repeopled from the few plucky survivors. Their descendants, *Iris setosa* var. *canadensis,* bear the scars of the glacial period, so to speak, in their conservatism; an innate invariability which, on the one hand, gives them a greater uniformity, and on the other, prevents their adapting themselves readily to other environments" ('36, pp. 495–6).

Cain ('40), in a recent discussion of supposed senescence in species, has accepted the ecological significance of biotype depauperations as outlined by Anderson. Instead of "conservatism" or "rigidity" in species, he calls the resultant phenomenon "narrower ecological amplitude." He is apparently doubtful, however, of the persistence theory for the explanation of isolated conservative species, and leans toward Wynne-Edwards' theory of habitat specialization.

Application of the Hypothesis. There is no place in the present paper for even a résumé of the details of Hultén's work, but it will not be amiss to draw from it a few examples, and to observe what he has done with some of the more striking boreal American problems.

Fernald's general thesis that cordilleran and arctic plants have persisted in the St. Lawrence region at least from a pre-Wisconsin dispersal is strongly supported. The relic plants are looked upon as "rigid" species, badly depleted of biotypes and now unable to spread rapidly. They occupied relatively small refugia and have thus been long separated from large populations of their kind. Their endemics are such isolated biotype variants within the ancient continuous populations as were sufficiently great to form taxonomic entities preservable either apomictically or through isolation.

The sharp contrast between these relic species and those which are "plastic" or "aggressive" is well-known to any one who has studied our boreal floras. The remarkable extent of coverage achieved by the aggressive types on the vast areas formerly denuded by glacial ice is about as notable as the fact that some of the plants have had no part in it. Whence came the vigor that made these migrations possible? All of the plants in the glaciated country must have been wiped out. They constituted the boreal flora of the last interglacial, and if they survived, their havens of refuge had to be on nunataks or outside the area covered. Why should some of these be so vigorous and aggressive while others are not? Hultén accounts for the difference very largely on the basis of relative sizes among the refugia. He conceives of two principal areas for persistence: "The Rocky mountains on the one hand and the continental shelf outside Eastern America on the other. From these centra the plants must have spread in an easterly and a westerly direction, respectively, towards the central part of the continent. . . . The fact that these plants have not lost their spreading capacity must be due to the extent of their refugia, which were so wide that the influence of the ice was not as devastating as in the north and as it was on the smaller refugia. In other words: they were not so severely depauperated of biotypes by the glaciation" (pp. 52–53). There is, by this reasoning, no necessary difference in age between conservative and aggressive species.

Two outstanding features of the eastern arctic flora have already been noted: its small percentage of endemism and the poor specific

boundaries among many of its species. Hultén, like Fernald, considers the arctic archipelago as having been a refuge for plants during the Pleistocene. He divides the plants of the eastern arctic into two categories, arctic and boreal, and makes a division of the former into rigid and plastic species. The smaller, arctic group are mostly regarded as radiants from northern Beringia, but since the extreme arctic species probably occupied the archipelago and its continental shelves not only throughout the last great interglacial but also the last glacial period, they did not suffer so great a reduction of their biotypes as if they had been confined to small refugia. Nevertheless, since they show a certain amount of progressivity as radiants from Beringia, and since there is so little endemism among them, they are thought to be very old, relatively rigid, and unable to spread far southward on account of their high arctic character. A less arctic group of North Beringia radiants does not go into the archipelago, but is continental in character. Some of these are thought to be of post-glacial origin;[5] while others are older and had achieved so wide a range in the last interglacial as to have reached lands south of the ice boundary.[6] The latter were thus able to maintain their spreading ability, and have now returned in force to their former areas in the glaciated territory. A few reached the eastern American refugia and were transformed into rigid types.

The boreal group in the eastern arctic flora is considered in two categories: arctic-montane, and boreal circumpolar. Both of these groups are among the most wide-ranging plants in the northern hemisphere, and have had ample opportunity both for isolation and freedom during their development. Both, however, possess characteristics which have tended to produce plasticity on a large scale. The arctic-montane group, through their montane connections, could preserve their migratory ability throughout the glacial periods to the south, and during the interglacials in the arctic. Since boreal-circumpolar plants "were not, like those surviving north of the ice, locked in between ice-sheets, they were not so depauperated and did

[5] In this group are such plants as *Arnica attenuata, Betula alaskana* (=*B. papyrifera* var. *neoalaskana*), *Cypripedium passerinum, Castillegia Raupii* (see also Pennell, '34), *Potamogeton Porsildiorum, Salix Richardsonii* (incl. *calcicola*), *Taraxacum lacerum, Primula egaliksensis, etc.*

[6] Here are some of the most wide-spread plants in boreal America: *Mertensia paniculata, Aquilegia brevistyla, Ribes hudsonianum, Festuca saximontana, Luzula saltuensis, Ribes glandulosum, Impatiens biflora, Cornus stolonifera, Orchis rotundifolia, Primula mistassinica, Picea canadensis* (=*P. glauca*) *Larix laricina, Picea mariana, etc.*

not to the same extent lose their ability to spread. It is natural, therefore, that the majority of the widespread plants of the arctic and boreal belts should belong to this group. The great diversity of races exhibited by these very plants is also quite natural, as their area has been filled up by radiants from several centra, isolated from remnants of an old area. This area must presumably have been occupied by taxonomically slightly different populations, which, after the isolation under selective pressure of the glacial periods, still further widened existing differences in different directions, parallel to the local conditions of the refugia. Few or perhaps none of the circumpolar boreal plants were so depauperated that they still exist only on the refugia, where they survived the glacial period, but many have not yet spread so as to completely fill up the gaps between them" (p. 120).

Hultén's work should provide a powerful stimulus to research in American phytogeography. Whether his ideas and conclusions are to be widely accepted or not, it must be admitted that they affect practically every geographic and taxonomic problem in the botany of this region. His views are the antithesis of those recently set forth by Wynne-Edwards, and he has elaborated the theme of differential ability to migrate far beyond the point reached by other workers such as Fernald and Marie-Victorin. His chief claim on the right to do this rests upon the recent genetical interpretation of the effects of isolation and population size.

(This article will be concluded in the next issue.)

THE BOTANICAL REVIEW

VOL. VII APRIL, 1941 No. 4

BOTANICAL PROBLEMS IN BOREAL AMERICA. II

HUGH M. RAUP
Arnold Arboretum, Harvard University

THE DEVELOPMENT AND DISTRIBUTION OF PLANT COMMUNITIES

Introductory—Static and Dynamic Concepts

It has already been stated that the description of plant communities in boreal America has lagged far behind that of the flora. Sparse though it is, the available material may be divided into two categories, depending upon the point of view from which it has been presented: an earlier "static" view and a later "dynamic" one. Chronologically, the division should have occurred in the early 1900's when dynamic concepts in the study of vegetation were being developed by such men as Clements, Cowles, and Transeau. Scarcely any application of such concepts to boreal American vegetation was seen until 1923 when Cooper published his first paper on the Glacier Bay region of Alaska.[1]

Sir John Richardson prepared the first general description of vegetation types for our region ('51). He proposed 5 provinces in northern America each of which had "a peculiar physiognomical character" in its vegetation: the "eastern woodland" (with its northwestern extension), the "Barren Grounds," the "prairie slope," the "Rocky Mountain chain," and the "lower woodland country on the Pacific side of the range." He worked out the main features of the northern boundaries of many common forest trees, as well as those of many other plants. Similar pioneer work was done in British Columbia by G. M. Dawson ('81*a*, '88) and John Macoun ('77), and in eastern Canada by Robert Bell ('84), A. P. Low ('06), and J. M. Macoun ('96). Except for the addition of some details, no further outstanding contribution was made until the Preble expeditions to Hudson Bay and the Mackenzie basin ('02, '08).

[1] The plant communities of Greenland are far better known than those in much of the Arctic Archipelago or the boreal parts of the continent. Some of the more important papers dealing with them are by Warming ('88), M. P. Porsild ('01), Sørensen ('35), Gelting ('34), and Böcher ('33b).

Preble applied the life zone classification of Merriam ('95, '98) to much of western and arctic Canada, setting off as Arctic all the country north of the limit of trees. The northern boundary of his Hudsonian Zone reached from the valley of the Liard River to Great Slave Lake, with an extension down the Mackenzie valley to about lat. 65°. South of Great Slave Lake it followed the eastern rim of the Slave River valley, crossed Athabaska Lake, and extended southeastward so as to pass to the south of Reindeer Lake. Thence it turned eastward to James Bay. Both Hudsonian and Arctic, of course, reappear on the higher mountains in the Canadian Zone to the southward.

It is unnecessary to describe in detail all the general classifications of boreal vegetation that have been published. Harshberger ('11) returned to a concept not far different from that of Richardson, recognizing tundra, prairie, an eastern forest region (Great Lakes-St. Lawrence region), a subarctic coniferous forest, Rocky Mountain forest, and northwest coast forest. Richardson had noted the change in character of the coniferous forests northwest of the Lake Winnipeg, but had not set up a separate province for them. Harshberger correlated his entire subarctic, northern coniferous forest with Merriam's Hudsonian, thus greatly broadening the concept of the latter as outlined by Preble. Weaver and Clements' recent map is essentially that of Harshberger, so far as boreal America is concerned, except that their northern boundaries for the Lake Forest and the grasslands are not so far northward ('38). Rydberg, in his discussions of Rocky Mountain vegetation, used the Merriam classification as a point of departure ('13–'21).

The Merriam system was utilized in recent years by Harper in his studies east of the Slave River ('31). Here he attempted to set up a faunal subregion between the Canadian and Hudsonian of Preble, which he designated as the "Tazin Highlands." This is considered a part of the Canadian Zone, differentiated from the Hudsonian by the temperature factor, and from the rest of the Canadian by a complex of climatic and edaphic factors.

The extent to which the Merriam system will remain useful in boreal America is uncertain. Its weaknesses for the classification of plant communities are legion as has been shown by Livingston and Shreve ('21). To adjust it to a dynamic viewpoint is particularly difficult. Western biologists have maintained it as a working

basis far more consistently than eastern students, probably because of the sharper biotic contrasts in the climatically and physiographically more diverse west. After sorting the whole flora of the Olympic Peninsula into life zones, Jones ('36, p. 17) found "that less than 5 per cent of the total number of species known to occur . . . show an anomalous zonal distribution." Jones has produced some illuminating results in his analyses of the floras of the Olympic Peninsula and Mt. Rainier by attempting a fusion of the Merriam system, Raunkiaer's classification of biological life-forms, and a dynamic viewpoint involving certain climax forest types in the Transition Zone ('36, '38). His comments on the Merriam-Raunkiaer combination are worthy of note: "The significance of the Raunkiaer system of life-forms as applied to the flora of the Olympic Peninsula is threefold. By means of this system a statistical analysis can be made of the flora of the whole region, or of the flora of each of the four life-zones taken separately. By whichever method used the results are comparable with the flora of other regions, and a simple but biologically sound summary of the phytoclimate thus can be obtained. As applied to Merriam's life-zones the Raunkiaer system yields valuable corroborative data. Merriam was concerned chiefly with the factors of climate which are effective during the season of growth and reproduction, whereas the Raunkiaer system is based on the adjustment of plants to the unfavorable, which is usually the dormant, season. By the application of both of these systems, a much clearer characterization of the life-zones or climatic formations may be obtained" ('36, p. 61). So far as the present writer knows, the application of such a scheme to any large part of boreal America has not been tried.

A recent and exceedingly useful map of Canadian forest regions has been drawn up by Halliday ('37). His basic scheme follows that of Weaver and Clements, recognizing three climax formations for Canada: tundra, forest, and grassland. The forests are subdivided into 8 regions or formations. "The recognition of such subdivisions is governed by the principles outlining the climax formation, and takes into consideration evolution and the relationship of species and genera. It is necessarily objective, indicating climate through the nature of the climax and reflecting it through the geographical position" (p. 6). A further subdivision into "Forest Sections" is "based on floristic considerations and the result of

physiographic, edaphic, and local climatic conditions" (p. 11). Within the sections some effort is made to correlate probable successional trends with the general character of the forest. Halliday's work is of particular interest for two reasons: first, it involves a far greater volume of information on Canadian forest types than has ever been brought together; and second, the basis for classification, though still static in many respects, contains the idea of development both in local situations and in terms of broad geographic entities. Through his division of "Forest Regions" into "Sections," he has found expression, in the region west of Hudson Bay, for the boundary between Hudsonian and Canadian noted by Preble. This boundary is a striking one to any one travelling across it, and was unrecognized in the more general classifications.

Certain phases of a developmental concept are to be found in the writings of Richardson. He reported, for instance, some evidence of recent changes in the position of the arctic tree-line ('51), and must have sensed the instability of many arctic and subarctic plant communities. Preble ('08) described the development of flood plain forests along the great northern Rivers, and John Macoun ('77) has a vivid description of the delta vegetation of the lower Athabaska and Peace Rivers. But the theory of vegetational succession has only recently been applied to whole sections of the plant cover.

The dynamic point of view has been a part of the reasoning in floristic plant geography for many years, dealing with the problems of glacial and post-glacial changes of climate and terraine. If common ground is to be found for these two methods of approach, the plant communities must, like the floras, be analyzed in terms of age, stability, and their relation to the events of recent geologic time. The patterns of distribution noted above, which have engaged the attention of vegetational geographers for so long, require dimensions for time and analyses in terms of species potentiality if they are to appear in their true perspective.

The evolution of plant communities on bare areas involves migration, establishment, competition, and the consummation of systems of communal interdependence. Current theories of speciation and of the inherent abilities of species to accomplish these things must also be taken in account. The analysis of boreal American plant communities therefore presents an endless series of problems which are

particularly intriguing because of the varying ages of the land surfaces and the probable varying potentialities among the species. Most of these problems are hardly more than sensed, much less stated and solved. Such beginnings as have been made in studies of the dynamics of boreal American vegetation will be summarized in the following pages.

Plant Formations of Boreal America

By far the greater part of boreal America is covered by two plant formations. The first of these is the Canadian coniferous forest, or Taiga, which extends from the Gulf of St. Lawrence to Alaska, and covers most of the slopes of the western mountains. North of this, and above it in mountainous country, is the formation commonly known as tundra. A third formation, of smaller areal extent, is represented by the natural grass lands of the central and southwestern parts of the Mackenzie basin which have their closest counterpart in the park lands of central Alberta and Saskatchewan.

The vegetation in all of this region was, beyond question, either completely destroyed by the Pleistocene ice, or highly modified if it managed to survive in unglaciated areas. It has acquired its present form, therefore, since the land surfaces of the north have become available, a process which has been progressive, and the sequence in which is still controversial. Except for the unglaciated areas, the sequence outlined by Antevs ('38) would place the oldest surfaces in the southeastern portion of our region, and the youngest in the Great Slave Lake country. Just how young the latter are is not yet known, but, as stated earlier in this paper, the matter is complicated by the great extent of post-glacial lakes. These lakes may have greatly reduced the interval available for the establishment of the modern plant communities. Likewise the effects of intense soil frost cannot yet be measured in terms of post-glacial time.

The Tundra. No adequate botanical descriptions, either floristic or phytosociological, of most of the treeless plains between the northern limits of forest and the arctic coast have ever been published. Many travellers have noted superficial differences among the types of vegetation to be seen in this vast area. There are moss and sedge tundra which are more or less wet throughout the year but there are also broad plains which are comparatively dry and covered with grasses and sedges. The region has been the princi-

pal grazing ground of countless caribou which still remain in enormous numbers.

Griggs ('36) has described the tundra in some detail in the Katmai district of Alaska, and has discussed the definition of the term "tundra" ('34*a*, pp. 157–9), showing that in a vegetational sense it is ambiguous, and should probably be used only in a geographical sense to cover all the vegetation of the treeless arctic. His discussion of Middendorf's concepts ('64) brings out the fact that the latter found some difficulty in differentiating, ecologically, the tundra and steppe of northern and central Asia. Similar problems will undoubtedly develop in boreal America. A more recent treatment of this question as it applies to Eurasia, and particularly in its historical aspects, will be found in a paper by Steffen ('38, pp. 177–95).

Porsild has published ('37*a*) a brief description of the tundra vegetation north of Great Bear Lake and west of the Coppermine River. He distinguishes first, heath associations over wide areas of rolling till-covered plain; second, meadows or prairies (of grasses and sedges) in the bottom lands between the low hills, "of greatest extent in the low alluvial plains bordering the Arctic ocean, west of the Anderson river, and in central Keewatin"; third, a sparse sand ridge type, relieved here and there by patches of green caused by manuring around ground-squirrel burrows; fourth, rock-face and rock-crevice types; and fifth, a rocky slope type. He considers that the meadows are "transitional," and are being invaded by the heath. Blanchet has also noted the contrast between heath and meadow types in the region northeast of Great Slave Lake, relating them to topography and soil ('26).

It is to be inferred from both Porsild's and Blanchet's descriptions, that the meadows are not necessarily stages in pond successions, but rather that many of them have developed upon relatively well-drained soils. Porsild suggests ('37*a*, p. 137) that "these plains were perhaps the last places to become available to plant life upon the recession of the Keewatin ice-sheet and the subsequent emergence of land from the sea and the draining of former lakes." Polunin has described some areas on Akpatok Island which are dominated by grasses, sedges, and other herbs ('34–'35). These occur in both xeroseres and hydroseres. Polunin also suggests that such vegetation is temporary, leading to a heath shrub type. Hultén

describes a comparatively narrow "alpine meadow" zone on the Aleutian mountains ('37*a*, pp. 37–39). He says it is being invaded from below by subalpine shrubs and from above by heaths which cover wide areas. "The heaths are found on all exposed places, while the meadows occur in more sheltered valleys or hollows between the small hills, ridges or plateaus."

The areal extent of these various types of tundra is unknown. It is probable that they have some broad correlation with major natural divisions of soil, topography and climate. Polunin has shown the importance of solifluction in determining the development of vegetation at Akpatok ('34) but its relation to the distribution of vegetation over wide areas, with diverse soils and climates, is still to be worked out.

The Forested Region. Although the Canadian forest is similar in life form throughout its area, it varies extensively from east to west in species composition, and from north to south in general aspect. In the east it involves mainly the white spruce, *Picea glauca,* and the balsam fir, *Abies balsamea.* In the Rocky Mountains *P. glauca,* represented to a large extent by western varieties, is associated with another fir, *Abies lasiocarpa.* The coast ranges are characterized by the western hemlock, *Tsuga heterophylla,* Sitcha spruce, *Picea sitchensis,* and giant cedar, *Thuja plicata.* In the far north and northwest the forests are largely reduced to *Picea glauca* or its varieties. Dry soils in the east and in most of the central part are dominated by the jackpine, *Pinus banksiana,* while those of the northern cordillera are characterized by the lodgepole pine, *Pinus contorta.* Muskegs or bogs are everywhere characterized by black spruce, *Picea mariana,* and larch, *Larix laricina.* Throughout this region the only deciduous trees of consequence are the white birches, *Betula papyrifera* and its varieties, and the aspens and poplars, *Populus tremuloides* and *P. tacamahacca.* From south to north, in the central part of our region, while there is some modification in species composition, a very noticeable difference is to be found in the general aspect of the forests. In the southern part they are relatively dense, with a rich ground cover of mosses and ferns. Near timber line they become open and park-like, with very little undergrowth or groundcover. The latter often consist of lichens and trailing heaths. The local distribution of these various types within the Canadian forest gives rise to many problems. They are not easily

arranged geographically, but seem to be interrelated in a complex manner due to present and past differences of soil and climate.

Within the forested region the most prominent vegetation boundary is coincident with the southern and southwestern margin of the crystalline rocks of the Canadian Shield. This was the boundary between the Canadian and Hudsonian life zones as laid down for the region west of Hudson Bay by Preble. To the east and north of the boundary the forest is greatly reduced in stature and density, and there are large areas of rock covered only by mosses, lichens, and such higher plants as can find space and soil in crevices and depressions. To the south and west the forests resemble superficially those of eastern Canada in having trees of good growth form, and a more or less rich cover of duff and humus beneath them.

In northern Alberta, northeastern British Columbia, and in the district of Mackenzie, these richer forests rest upon rocks of Paleozoic or Cretaceous age. Most of the Cretaceous rocks here exist in the form of erosion plateaus, isolated, or toward the southwestern part of the district, more or less continuous and forming the high plains of north central Alberta. The northern boundary of the continuous Cretaceous plateau marks another vegetational and floristic province. There are modified recurrences of it farther north on the isolated Cretaceous plateaus mentioned above. As noted previously, the more mesophytic, eastern Canadian forests are dominated by white spruce and balsam fir. As one passes northwestward through these forests the fir disappears at approximately the northern boundary of the Cretaceous plateau. Many other species also disappear in the same way, so that the far northwestern coniferous forest becomes greatly simplified in species composition, and the white spruce *Picea glauca,* becomes the most important tree except on very light soils or in muskegs. In the Mackenzie drainage basin the boundary of the Cretaceous plateau lies on the lower Athabaska and Peace Rivers. The balsam fir has been found in the Athabaska River delta, but north of this point it is extremely rare or nonexistent. Records of it are in the literature as reappearing on the Mackenzie and near the sixtieth parallel west of the Slave River, but neither of these records has ever been verified. Many of the species which disappear at the northern margin of the plateau are to be found far westward in the Rocky Mountains and some even northward in the cordilleran region to Alaska, but they have never been

found in the comparatively simple forest of the lower and central parts of the Mackenzie basin (Raup, '30).

To the southwest the margin of the Cretaceous plateau continues to be a significant boundary, although with different species involved. Here a cordilleran influence is felt, with the northeastern extension of such trees as the lodgepole pine, *Pinus contorta* var. *latifolia,* and the balsam fir of the Rocky Mountains, *Abies lasiocarpa.* Many other characteristic cordilleran species accompany them.

The contrast in soils between the pre-Cambrian and younger formations is striking, and sufficient to account for such wide vegetational differences as occur. Edaphic differences between the Cretaceous areas and the lower, Paleozoic surfaces, most of which are till-covered, are not so clear. The present writer ('30) has tried to account for the absence or rarity of many species in the lowland and northern forests of the Mackenzie basin by postulating extreme immaturity in the forest communities and soils. "The most profitable line of inquiry appears to be in the edaphic factors of the environment, especially in so far as they are affected by the rigorous climate and the short time available since much of the vegetation had its inception" ('30, p. 207).

Further anomalies appear when the "simple" forests of the lower, northern country, both on Paleozoic and pre-Cambrian formations, are analyzed. The ultimate in simplification is reached in some of the jack pine woods on dry sandy soils about Lake Athabaska. Here is rather open timber whose trees are 70 years old or more, with a close ground cover of lichens. After careful examination of a large area only half a dozen vascular secondary species could be found; and there are whole acres whose only vascular plants are the pines.

The white spruce forests occur in three phases which are geographically rather distinct. On the great river flood plains the trees grow to large size (75–100 ft. high, 2–3 ft. in diameter), and in close, nearly pure stands. The undergrowth is relatively thick, but the ground flora is sparse, with a rather thin carpet of mosses. Stages in the development of this type are to be seen everywhere in the flood plain and delta country, and have been described in some detail (Raup, '35). Closely related to the forests on alluvial soils are those on the better drained glacial soils of the uplands. Here the stands

are not quite so dense and the undergrowth is thinner. The ground is covered, however, with a mat of mosses and duff 4–8 inches thick. Some of these forests appear to have developed from a jack pine type (Raup, '33*a*, '35), but others may have come from a third kind of spruce wood. This third phase is quite different from the other two, and consists of an open, park-like stand of timber with practically no undergrowth. The ground is usually covered with a lichen-heath mat, and the soils are light and sandy. This type is best developed in the pre-Cambrian parts of the region, but representatives of it are occasionally met with in the country farther west and south. It is the spruce forest that appears at arctic timber line.

No specific differences among the spruces of the three types have been found. It is not impossible that there are some racial variations whose outward manifestations are physiological rather than structural. We have to deal, therefore, with a single species which forms and dominates, side-by-side in the same region, three different kinds of forest communities, all of them with different origins and stages of development. Here, apparently, is a wide range of tolerance in the white spruce, brought about by a system of controls and releases about which we know almost nothing. The species is not far from the margin of its range; and as Griggs has pointed out repeatedly ('14, '34*a*, '34*b*), the solution of many of our geographic problems may hinge upon more intimate knowledge of the behavior of plants under such conditions.

Working upon the hypothesis that there has been only an amelioration of climate since the land surfaces became available, the present writer has suggested ('33*a*) that the earliest post-lacustrine and post-glacial forests were of white spruce arranged in an open, park-like association similar to the third type noted above. With the warming of the climate and the decrease of ground frost the less tolerant jack pine could migrate into some parts of the country, where it could compete successfully with the spruce on the dry sandy soils. An increased growing season, more available moisture, and more rapid decay then permitted the accumulation of humus; and a forest succession was set up by which the spruce followed the pine again to form the second type. The youngest of the three is on the new flood plains at the lowest levels. Remnants of the original open spruce type still persist within the areas of the other two, on sites which the pine cannot invade due to local conditions. Some support

for this concept is to be found in Pulling's studies of root flexibility among northern trees ('18).

Some evidence for the immaturity of boreal vegetation in the glaciated regions is to be found also in pond shore communities. During the writer's botanical exploration in the Athabaska-Great Slave Lake district, notes in more or less detail have been made on some 70 ponds. In the pre-Cambrian parts of the district 161 species of vascular plants were listed in these habitats. No less than 58 of them (36%) were put down as primary species in at least one association, and they appeared in 83 different associations within which they dominated. This lack of uniformity and continuity in the distribution of primary species is one of the most striking aspects of the pond vegetation of the region. The writer makes no pretense to having exhausted the possibilities, and he suspects that the examination of more ponds would only increase the number of combinations to be recognized. It looks as though the marginal communities of the ponds were just becoming aggregated out of migrating species. Even if a uniform series of pond habitats were available, a wide variability among the communities could be expected on this basis alone.

With such figures as those just given it has been impossible to set up any kind of successional series applicable to more than a very few ponds. The only organization of the data which has so far proved feasible is on a geological basis. There seems to be some correlation between the distribution of species and communities, and that of the major rock formations. In fact only 10 species could be listed in all of the geological divisions set up.

Subarctic Prairies. Another set of problems which has appeared in the study of the forested country pertains to the semi-open prairies which extend from the Upper Peace River far to the northeastward. They are known to occur throughout the Lower Peace River country, to have large extensions in the Wood Buffalo Park west of the Slave River, and are thought to have representatives on the northwest side of Great Slave Lake and west of the upper Mackenzie. These prairies have been studied in a few places on the Upper Peace River (Raup, '34), and in the southern part of Wood Buffalo Park (Raup, '33*b*, '35), but otherwise they are yet to be investigated. Their presence scattered through nearly the entire breadth of the forest zone is a somewhat anomalous situation, since the belt of coniferous forest is generally regarded as a climatic for-

mation, and the general conditions of temperature and rainfall throughout the year suitable for the growth of trees. The influences determining the presence of this semi-open prairie country are, therefore, yet to be determined.

The "Chinook" winds of Alberta, northeastern British Columbia, and western Mackenzie create rather distinctive local climates in those regions (Raup, '34). They might be related to the occurrence of the prairies, although it can be shown that some of the prairies extend far outside the probable range of effectiveness of the "Chinooks" (Raup, '35). If these winds are to be effective in keeping down tree growth, the damage is probably done by winter-killing, since the winds are a feature of the winter climate. Local foehn winds have been noted elsewhere, such as on the north shore of Lake Athabaska, but their biological significance is unknown.

Certain essential facts concerning the prairies are: first, so far as they are known at present, they are confined to dark loamy soils of post-glacial lacustrine origin; second, they occur on land surfaces which have a fairly efficient surface drainage; third, the grass land seems to represent a virgin type of vegetation on the soils which it occupies, using the term "type" in a broad sense; fourth, aspen and occasionally spruce forests are slowly encroaching upon the prairies although in many places but little progress has been made; and fifth, grassland soils on the uppermost of the ancient lake bottoms show the most advanced (chernozem) development of soils (Raup, '34).

Floristically the prairies of the central and southern parts of the Mackenzie basin closely resemble those of central Alberta. But those still farther north appear to be different, involving fewer xerophytic grasses and more sedges. One of the most intriguing botanical problems in the north is the discovery of the floristic and structural relationship between these northern grasslands and those of the arctic tundra. Is there an unbroken series of transition types that will give us clues to the formation and history of all our prairies? And if so, what will be the evolution of soils under such a series?

It is suggested that the Mackenzie basin prairies developed as such directly upon the new soils exposed at the drainage of the post-glacial lakes, or that they followed some sort of tundra which in turn originated on these soils. (Raup, '34, '35.) At least two other interpretations might be advanced. It was originally thought that both the Peace River prairies and those of much of the park land of

central Alberta and Saskatchewan were caused by fire. It was supposed that repeated burnings of the timber during dry periods kept the grass lands open. Recent studies by Moss, however, in central Alberta ('32) have shown that all of the aspen park land and the so called poplar belt have developed on dark soils which must have had their early history under grass land. Moss has shown that fire can be reckoned only as a deterrent in the development of timber on what were originally grass lands. A second interpretation involves a post-glacial warm period during which prairies may have pushed northward, to be later cut off by a deterioration of the climate. There is yet no other evidence in the region to support such a theory. The study of peat deposits may throw some light on it, but no remains of forests have yet been found in the prairie soils. It must be emphasized again that our post-glacial chronology for this region is not comparable with that of many other parts of the glaciated country, and that such a climatic optimum may have occurred before much of the present land surface was exposed.

Timber-line Problems

The position of the tree-line, its causes, and its possible fluctuations have been studied in a few places, and commented upon by many travellers. On the older maps it was shown by a fairly even line; but with increasing information the boundary becomes more and more ragged. There are long northward extensions down the valleys of the Coppermine, Thelon, Dubawnt, and Kazan Rivers, with isolated areas in the upper Lockhart basin and upper Back River valley, and in the country between Point Lake and Bathurst Inlet (Halliday, '37). Clarke has recently described its convolutions in the country east of Great Slave Lake ('40). Whatever its causes, the northern limit of trees is a biotic boundary of major significance, equivalent in its way to the transition from forest to prairie in middle latitudes.

Griggs presents rather convincing evidence that the timberline in Alaska is moving into the tundra: "(1) All the trees near the edge of the forest are young—less than 100 years old, whereas three miles back from the edge they exceed a meter in thickness and are over three hundred years old. (2) Many old trees now in a dense forest of younger growth are 'open ground' trees with branches, now killed by over-shading, clear to the base. (3) The rate of growth at the

forest edge compares favorably with that of the same species, Sitka spruce, in southeastern Alaska many hundred miles behind the edge. (4) Records left by early Russian settlers explicitly describe as treeless, areas now covered with heavy forest. (5) Peat from the bogs contains only a few scattering grains of spruce pollen such as would be blown a long distance, thus demonstrating that the present is the first forest that has occupied the ground since the beginning of the bogs, *i.e.*, since the glacial period" ('34*a*, p. 163). Griggs also quotes Robert Marshall, who studied timberline conditions in northern Alaska, to the effect that the youngest, and outermost trees at the edge of the tundra have as good a growth rate as those many miles to the southward. Several other observers have reached similar conclusions for other parts of Alaska (see Griggs, '34*b*).

Using this apparently active advance of timberline as evidence, Griggs concludes that it is not now a climatically determined boundary in Alaska although timberlines generally are thought to be climatically fixed. He suggests that the timber is far behind an amelioration of climate that took place sometime ago, and is now finding no great difficulty in invading the neighboring tundra.

The northern forest boundary east of Hudson Bay has not been studied so extensively at any point as it has been by Griggs in Alaska. Nevertheless it is probable that here the line is retreating instead of advancing. Abbe has reviewed the scanty data on recent changes of climate in northern Labrador and Greenland ('36, '38), citing the excavations of old Norse colonies in the latter region (Nörlund, '24). There seems to be incontrovertible evidence that beginning in the latter part of the 10th century a deterioration of climate began in southwestern Greenland. It did not become serious until the latter part of the 12th century; but after that the Norse colonies rapidly declined under the influence of increasing cold, and finally perished. The present writer ('37) has gathered evidence from the fields of botany, zoology, paleontology, and archaeology which strongly suggests that there has been a deterioration in the climate of New England within the past 3000 years or less. Fernald found some indication of a retreat of coniferous forests at the Straits of Belle Isle ('11). It is therefore not unreasonable to expect that shifting of the tree-line in the east has been in the opposite direction from that in Alaska.

Between Hudson Bay and the Mackenzie delta, on the other hand,

evidence of change is variable. J. B. Tyrrell, one of the keenest and most widely travelled observers ever to visit the north, could find no evidence of anything other than an amelioration of post-glacial climate in the regions east of Athabaska and Great Slave Lakes ('10*b*). He found many dead trees in the outer northern groves, but noted that most were alive. The absence of *Sphagnum* bogs in the tundra districts was used as evidence that forests had never grown there, since these bogs are now limited to the forested regions. Clarke has recently published what appears to be an exhaustive study of the timber line east of Great Slave Lake, and finds it relatively stable ('40). "Many traces can be found of clumps that have perished, leaving only bleached sticks to tell the tale, and there are others obviously new. On the whole there seems to be a very slight gain in these clumps more often than the contrary, and the writer believes that they are not sterile" ('40, p. 22); and "The Thelon must have looked much as it does now for hundreds of years, and similar climatic and physical conditions have prevailed for a couple of thousand years anyway" (p. 21). Clarke thinks that the line is now actually to be correlated with existing climatic phenomena, rather than by historical interpretation as Griggs found necessary in Alaska.

Richardson found evidence on the lower Coppermine River that the forest line was retreating southward ('51). This was mainly in the form of many isolated clumps of dead trees scattered over the "barren grounds," unaccompanied by any living ones. He made the significant observation that while the living clumps were confined to sheltered places, many of the dead ones were on exposed hillsides where they could have grown only if the climate were more congenial. Johansen has confirmed this observation ('19, '24), also maintaining that there has been a recent deterioration of climate. Porsild presents some evidence from the Mackenzie District, as follows: "We have no proof that the climate of the Mackenzie District is becoming colder or that the limit of perpetually frozen ground is extending downward, but we have botanical evidence favoring this view. On Richards Island, for example, 60 or 70 miles north of the present limit of trees, well preserved roots and stumps of a former spruce forest are found *in situ*, now covered with a peat deposit many feet thick. In peat deposits presumably of still greater age, on the east branch of the Mackenzie Delta, the writer discovered

larch cones more than 50 miles north of the present range of this tree. *Potamogeton epihydrus* var. *Nuttallii,* discovered in the peat deposits in Pingorssarajuk, is not now found anywhere in the Northwest Territories.

"As regards recent botanical evidence, there are in the Eskimo Lakes basin isolated occurrences of several boreal or even temperate species such as the duck weed (*Lemna trisulca*), water arum (*Calla palustris*), yellow water lily (*Nymphozanthus variegatus*), sedges (*Carex norvegica* and *C. petricosa*), *Agoseris cuspidata,* pondweeds (*Potamogeton pectinatus, P. Friesii*), *Lappula Redowskii,* and several others that may be considered relics from a milder, postglacial period" ('38, p. 57).

To sum up, the tree line seems to be advancing in Alaska, retreating in northwestern Mackenzie, approximately stable in southeastern Mackenzie, and apparently retreating in eastern Canada.

The northern limit of trees is generally thought to be determined by climate, whether in an immediate sense or on a broader historical basis. Observers in the interior of the continent are agreed that exposure to cold dry winds, particularly those with a northern component, is a determining influence at the tree line: Porsild ('37*a*), Blanchet ('26, '30), Clarke ('40). Clarke thinks that outliers of timber are in part edaphically determined, growing in sheltered positions, on soils that are unfavorable to the tundra type. Just why the line should be where it is, however, is not clear. Blanchet has expressed the problem quite clearly: "It is difficult to conceive of a line drawn across a plain on one side of which trees grow while on the other they cannot. However, such a line does exist, modified by variations of soil and shelter. . . ." ('25). There is a rough correlation between the tree line and the July isotherm for $+10°$ C., as noted previously (Nordenskjold, '28), but Griggs has pointed out the rather serious discrepancies that appear when the matter is examined in detail ('34*a*, p. 161). According to his figures the forest is 250 miles below the isotherm on the Alaska peninsula, about on it in northeastern Alaska, nearly 150 miles north of it east of the Mackenzie, 350 miles south of it on the west coast of Hudson Bay, and nearly 400 miles north of it again in northern Labrador. Recent climatic maps (Brooks and Connor, '36) greatly reduce the discrepancy on the west coast of Hudson Bay, and eliminate most of it in northern Labrador.

Stupart ('28) has suggested that in northeastern Canada (Labrador Peninsula) the northern limits of trees and agriculture may be correlated with the position of the country in reference to the mean path of cyclonic storms. "Over all this region the prevailing winds have a northerly component in summer as well as in winter, this owing not to abnormally high pressure in the north, but to the fact that in all seasons the mean path of cyclonic areas lies to the southward of this region," and "In the northern portion of this northeastern territory there are vast tracts of land where the climate is so completely dominated by Arctic influences that the country is treeless and agriculture is impossible" ('28, p. 41). Maps of the tracks of cyclones have been published by Connor ('38) for boreal and north temperate America, and by Shaw ('36) for the circumpolar area. An earlier map of circumpolar storm tracks was published by Loomis ('85). So little is known, however, of the actual arrangement, both in time and space, of these major influences that to draw up such a broad correlation with the tree line as is suggested above would be hazardous.

A series of challenging problems should be found in attempted correlations between changing climates and vegetations on one hand, and on the other the migrations of aboriginal populations. Recent papers by Jenness ('33, '37) have described extensive migrations, among both northern Indians and Eskimos, during the Christian era. He divides the existing Canadian Eskimos into three groups: (1) those of the Mackenzie delta (and formerly of Southampton Island); (2) those on the treeless plains west of Hudson Bay; and (3) those of the arctic coasts from Coronation Gulf to Labrador. The first are considered to be descendants from an ancient ("Thule") people who migrated from Alaska "to the eastern Arctic about 1000 or more years ago, dropping colonies all along their route." The second group are known as the Caribou Eskimo, and are the remnants of a "second great reservoir of the race—the inland Eskimo, now shrunken to a fast vanishing pool." The third group, as it now exists, "flowed out of this inland reservoir about 1200 A.D., overwhelmed the earlier coast-dwellers, and in their new environment gained a fresh lease of life and vigor."

The Indians of the northwestern interior of Canada and Alaska are of Athapaskan stock. They have always been a race of non-agricultural, land hunters, having very little contact with the sea,

but utilizing fresh-water fish. With but few exceptions they have stayed within the boundaries of the forested country. Jenness has outlined their migrations as follows: "If now we contemplate these late movements of the Athapaskans, and the distribution of their tribes at the opening of the historical period, we seem justified in assuming that at the beginning of the first millenium A.D. they were still massed in the northwest corner of the continent, but had already begun their southward trek. One division occupied the basin of the Mackenzie river to the edge of the Barren Grounds, where the lack of timber halted them, and perhaps also the hostility of the inland Eskimo. It was partly the lack of timber, too, that checked their expansion onto the prairies, for even in later times the Sarcee clung to the forest border until they obtained horses from the Blackfoot. Most of the migrants, however, did not cross the Rockies, but drifted far down the western plateau, travelling, like the early Navaho, in small bands of two or three families that here and there, in favorable localities, concentrated to form new tribes" ('37, p. 36).

Causes for these migrations have not been determined. Dr. Jenness suggests fluctuations in the supply of game, wars over tribal hunting grounds, and a sheer spirit of adventure. To anyone thinking in terms of vegetational change, the question naturally arises as to whether coincidences might be established between the two series of events. Two groups of people are involved, Indians and Eskimos, whose cultures have been closely related, respectively, to the wooded country and the tundra, and whose separation has been, from time immemorial, at the arctic timber line. Could it have been a northward movement of the forest border that started the inland Eskimo toward the coast about 1200 A.D., either by narrowing their hunting grounds or by increasing the pressure from migrant Athapaskan peoples to the south and west? Again, is it possible that the movement of Athapaskan Indians southeastward from Alaska was coincident with the actual arrival of forests in the lower Mackenzie region and the northern Rocky Mountains?

We are woefully lacking in vegetational chronology for the regions in question. About all that can be said now is that the timber line shows a tendency to fluctuate (at an unknown rate), and that the plant cover as a whole appears to be very "young" and "immature." Some evidence for the youth of the vegetation is noted elsewhere. It should be remembered that the time interval is not a post-glacial one, but rather a shorter, post-lacustrine period.

Aboriginal legend, unless it can be checked from other sources, is a notoriously unsubstantial basis upon which to build history. Nevertheless a few legends that involve changes of climate and biota in western Canada are worthy of note. David Thompson's Indian guides, while travelling with him through the Athabaska Pass in 1811, expressed a firm belief in the existence of the mammoth, a tundra animal, in that region ('16, p. 445). The Eskimo have myths and a word in their language for the mammoth (Jenness, '24). By far the most interesting story that has yet come to light, however, was reported by Mackenzie, in 1793. Mackenzie was at his winter quarters along the Peace River near the mouth of the Smoky, and one day interviewed an old Indian who tried to tell how old he was. "An Indian in some measure explained his age to me, by relating that he remembered the opposite hills and plains, now interspersed with groves of poplars, when they were covered with moss, and without any animal inhabitant but the reindeer. By degrees, he said, the face of the country changed to its present appearance, when the elk came from the East, and was followed by the buffalo; the reindeer then retired to the long range of high lands that, at a considerable distance, run parallel with this river" ('01, p. 143). It is hardly conceivable that this Indian was actually relating personal experience; but it is not impossible that he was drawing upon tribal history that was sufficiently recent to contain considerable detail. The change from tundra to grass land described by him in the Peace River region is precisely that which recent studies of the prairies suggest (Raup, '34, '35).

Ground Frost

Most of boreal America has a permanently frozen subsoil, the botanical consequences of which are far-reaching. Data on the depth to which the soil is frozen and the amount thawed each year are exceedingly meager. Harshberger ('28) and Cooper ('23, '37) have noted some features of the problem in Alaska, and the present writer has discussed some of the effects of frozen soils in the Mackenzie basin ('33*a*, '35). Porsild has noted that the ground in unglaciated parts of Alaska is not frozen to as great depths as it is in most of the north ('38). Pulling's studies in northern Manitoba ('18) are of particular interest because he attempted an actual correlation of the root-habits of trees with frozen subsoil. Porsild's in-

vestigation of "Pingoes" in western arctic Canada should also be noted ('38). Smith ('39) has a discussion of permanently frozen ground in Alaska in his paper on the areal geology of Alaska, with a map of localities from which data are available. There is also a map of the extent of Quaternary glaciation in Alaska (see also Capps, '32).

Light sandy soils may have no permanent frost in them, or they may thaw to great depths each year; while immediately adjacent, heavier soils remain frozen near the surface. The prevalence of muskegs or bogs in the north is due in many cases to impervious frozen strata beneath the surface. The wide-spread uniformity in muskeg floras may be ascribed at least in part to the abundance of such habitats. The maintenance of so much ponded water has undoubtedly served to retard the processes of erosion even further than they are already retarded by the shortness of the open season. Thus the maturing of stream drainage systems, even in comparatively friable materials, is slowed down; and all the resulting developmental processes in the vegetation are correspondingly slow. No measure of these retarding influences has been found, nor has it been possible to calibrate them in terms of past events.

Pulling has set up a classification of the common northern forest trees on the basis of their inherent flexibility of root-habit. Black spruce, tamarack and canoe birch are regarded as having a "rigid shallow root habit"; white spruce a "flexible shallow root habit"; balsam poplar a "deep flexible root habit"; and jack pine and white pine a "deep rigid root habit" ('18). Pulling suggested that the northern extent to which these trees might go was determined by their ability to live in soils made shallow either by frost or by the thinness of deposits over much of the Canadian Shield. The present writer has utilized the classification in accounting for some features of forest distribution in the Mackenzie basin ('33*a*); but a wider application of the idea, to embrace other elements of the woody flora, would no doubt prove worth while.

The difficulty of studying the rates of vegetational change in relation to stream erosion and deposition, previously discussed, applies also to lake and pond shores. The open season during which geomorphic processes can operate is much shorter than in more southern climates, and it differs greatly within the boreal region itself. We speak of the vegetation of the central Mackenzie basin as "young,"

perhaps "very young" if Antevs' theory of a late rejuvenation of the Keewatin ice is accepted and we take into account the length of time the post-glacial lakes occupied the country. But if the extreme slowness of decay, humus formation, and physiographic processes are considered, the "youth" may be a qualitative rather than quantitative concept. The development of vegetation in a subarctic pond, for instance, or on a receding lake shore, may be rendered so slow by the rigorous climate that the succession effective in more temperate regions are not applicable. It may be necessary to interpret the ordinary stages of development with time intervals so long as to extend over periods of climatic change great enough to change the facies of all the plant associations.

The effects of frost action upon surface soils were noted by Harshberger ('28) as a fruitful source of problems in arctic botany. Their significance in modifying plant communities in the tundra has also been suggested by Griggs ('34*a*). The most extensive studies in the American arctic, however, are probably those of Polunin at Akpatok Island, in Ungava Bay ('34–'35).[2] Here a large part of the surface of the island is characterized by "polygons" of limestone fragments or finer material which are so constantly churned by the action of frost as to be almost sterile of plant life. Only on the more stable portions can a handful of plants find a chance for survival. The geographic and historic meanings of this phenomenon have not been worked out. Although soil polygons and other evidences of intense frost action have been described in many parts of the arctic, yet their actual surface distribution is yet to be outlined and related to other geographic features.

Polunin gives a detailed structural description of the ones he saw, and reviews some of the theories advanced to explain them. It is quite obvious that they occur most abundantly in arctic and alpine climates, and that the availability of moisture and the occurrence of certain types of rock are important conditioning influences. Recent studies by C. S. Denny on central New England mountains ('40) indicate that "the factor which controls the development of stone-rings on mountain summits is the presence or absence of vegetation, provided that suitable rock basins filled with relatively fine debris (till) are also present . . . therefore stone-rings should be found on those summits which are treeless." Other studies on the New

[2] Investigations along this line in Greenland are described in papers by Sørensen ('35) and Seidenfaden ('31).

England Mountains by Antevs ('32) and Goldthwait ('39) have not only defined present day frost-action effects, but also larger "fossil" polygons and stone stripes. The effects of intense frost action have been found to be widespread in southern New England and adjacent New York (Denny, '36, '38; see also Bryan, '28), in regions now in temperate climate and under forest growth. That these last are "fossil" effects, dating from a time of treelessness and arctic climate, has not been conclusively proved though the evidence on the ground, as well as the studies by Denny noted above, point rather clearly in that direction.

From these facts it seems clear that in the arctic and alpine regions of boreal America the manipulation of surface soils by frost is now a phenomenon of considerable significance, and that it may have been significant over much of the country at an earlier time. Its effect upon modern arctic vegetation has been described by Polunin ('34–'35), who considers that this habitat on Akpatok Island is one of long-standing and may continue for a long time: ". . . the climatic, topographic and edaphic conditions on Akpatok are all very unfavourable to plant growth and have combined to keep the vegetation extremely scanty and reduced. These conditions have probably obtained much as they are today for at least one or two thousand years, and there is every reason to suppose that during most of this time the plant communities, being in the majority of cases too miserable to accumulate humus, have also remained substantially unaltered." Studies in temperate alpine regions also strongly suggest that vegetation is closely related to frost action; but here the point of view is reversed, and it is thought that the presence or absence of trees determines whether there shall be frost-heaving or not. Both views are probably correct, and there is a point in the transition between arctic and southern climates where vegetation can actually overcome the churning by frost and finally stabilize the soil.

Another suggestion that comes out of this field of inquiry is that in any historical-vegetational chronology we set up for the glaciated parts of our region, it may be necessary to insert an indefinite interval during which plants could scarcely make any headway at all. This would be the case particularly on soils and surfaces suitable for frost action. Polunin found it difficult to visualize any successional changes on most of Akpatok Island except those that had occurred when the land was first exposed at the retreat of the ice. He set up what he calls a "polygon subclimax" in order to place the vegeta-

tion in a hypothetical sere. Theoretically the polygons on Akpatok should eventually be reduced entirely to fine materials, but the vegetation that would then grow on them is conjectural.

In any case, one of the most fruitful fields for botanical research in the north should be this inquiry into the effects of ancient or modern frost action. The surface evidence of solifluction is not always easily recognized by the uninitiated. Consequently the fact that it has not been reported in a region does not indicate that it isn't there. Larger stone polygons are readily recognized but small, temporary stone-rings and stone-stripes, as well as the cumulative effects of small disturbances upon slowly moving slides will be recognized only with practice.

Other frost action phenomena, perhaps of lesser contemporary biological significance, are the "earth mounds" of the western arctic. These are so common and striking in appearance that there is a term for them in the Eskimo language: "Pingo," meaning conical hill. They occur, according to Porsild ('38), "On the otherwise low and featureless coastal plain . . . from Point Barrow eastward past the delta of the Mackenzie River to the first outcrop of rock east of the Horton River." Other mounds have been found on the Seward Peninsula and in the Kotzebue region of Alaska. Porsild has examined a great many in the field and has reviewed the theories advanced to explain them. Some, situated on sloping country where there are previous layers of gravel, may be due to hydraulic pressure. Others, in level country, appear to be "formed by local upheaval due to expansion following the progressive downward freezing of a body or lens of water or semifluid mud or silt enclosed between bedrock and the frozen surface soil, much in the way in which the cork of a bottle filled with water is pushed up by the expansion of the water when freezing" ('38, p. 55). The latter type always occurs "in or near the border of a lake or in the basin of a former lake," and has in the summit a crater-like depression the sides of which show the stratification of the lake bed in which the mound has been formed. Porsild has shown that these fractured and exposed surfaces are excellent places to collect peat samples which should yield valuable evidence of earlier vegetations and climates.

Peat Problems

A promising field of research in boreal vegetation is the study of peat deposits. Bowman has published the results of his pollen

studies at Kodiak ('34), and Cooper has investigated the fossil forests at Glacier Bay ('23, '31*a*, '37, '39). Porsild has pointed out the possibilities of such studies in the lower Mackenzie region ('38), and Tyrrell has published valuable notes on the general distribution of peats in the country west of Hudson Bay ('10*b*). Erdtman ('31) has published the results of some studies in the peat bogs of central Alberta. It is safe to say, however, that the field is entirely open, and holds unusual promise as a source of major contributions in boreal American phytogeography. It should also be of value in the solution of many perplexing physiographic problems.

There are large islands in the western part of Great Slave Lake, near the entrance of the Mackenzie River, which seem to be composed entirely of peat. The lake waves have cut terraces in the peat that afford excellent sections from which to take samples. The origin of the deposits is conjectural. If our present concepts of lowering lake levels are correct, then it is possible that during the stages immediately preceding the present there was an immense bog in this western part of the lake which has been excavated by wave action as the water receded, leaving only a few remnants.

If peat analysis in the north is to be effective it must be done with a clear understanding of the physiographic problems involved. It should be remembered that the sequence of post-glacial geomorphic events is only sketchily known. In the Mackenzie basin an excellent starting point would be the existing theory of lake expansion and contraction, with the realization that at best this is only a working hypothesis, subject even to radical changes as new facts appear. Likewise it must be borne in mind constantly that our vegetation boundaries and climates are not stable, but have fluctuated widely in the past, with results which are yet obscure.

Methods and Concepts

Associations and Successions. The ecological description of boreal vegetation bristles with problems of method and concept. Analyses in terms of plant associations, or attempts to arrange the associations in seres, often become so complex and involved as to defeat their own purposes. Griggs, in trying to describe the arctic plant cover of the Katmai region in ordinary ecological units, found it hopelessly complex. "In the temperate zone vegetation is rather clearly segregated into more or less well-marked associations, like

beech forests, oak forests, pine woods, swamps, and bogs. . . . When one goes to the arctic he naturally expects to find similar plant associations, but instead he meets a bewildering mixture of plants of all sorts jumbled together in seeming defiance of the principles of plant association learned in low latitudes" ('34*a*, p. 154). He stresses repeatedly the difficulty of setting up a group of associations with which to generalize over any large area, pointing also to the experience of Scandinavian botanists who have set up a multiplicity of associations to describe the occurrence of a very few species. (See also Faegri, '37*b*, for a recent review of European problems in this connection.)

The present writer has had similar difficulties in the forested regions of the interior. The necessity for describing three kinds of white spruce forest in the same district is anomalous in the light of our rather well-defined temperate types. Again, the failure to find any phytosociological basis for generalization among pond floras is an excellent example of the same kind of confusion. Polunin described thirty-odd combinations involving vascular plants on Akpatok Island, with a total vascular flora of only 129 species ('34–'35). He notes particularly the floristic uniformity of the vegetation mixture, and makes no attempt to outline communities until he has described a series of special habitats in which to look for them. A glance at the designations he uses for many of his communities indicates the difficulty he had in defining them: "Carex-grass-forb mat," "Dryas, etc. Fjaeldmark," "Dryas-Salix-herb polygon sub-climax," or just "Forbs." Hultén apparently found similar difficulties on the Aleutian Islands.

Griggs' ('34*a*, p. 174) conclusion is that "Each of the items contributing to the belief that arctic vegetation remains in a state of flux goes to indicate that the plants of the arctic, individually and collectively, are still far from equilibrium with their environment." He thinks that the arctic vegetation "has not yet recovered from the glacial period but is still in process of active readjustment." This is consistent with the writer's own findings in the Mackenzie basin.

Griggs reaches still another significant conclusion: that "a science of arctic ecology cannot be built up on the assumption that the place and mode of occurrence of a plant give reliable indications of its optimum habitat." Here he is in essential agreement with several other students whose work on relic species and communities is reviewed in this paper.

With so much confusion in the *content* of northern plant associations, their organization into developmental series, especially under the existing climatic and physiographic conditions, becomes equally obscure. The difficulties in working out the history of what look like comparatively simple seres on the shores of large northern lakes has already been discussed (pp. 23–4). Similar studies in ponds, alluvial deposits, and upland forest seres suggest that the successions are not conditioned so much by biological factors as by climatic and edaphic ones. That is, the presence of a plant association in a given place is dependent not so much upon the previous existence of another one which prepared the ground, as upon the availability of the ground itself and a suitable climate. For instance, it is suggested that the Banksian pine has invaded the park-like spruce forests because a lowering of the upper limit of ground frost made possible the penetration of the rigid taproot of the pine, rather than because the spruce had modified the soil so that it could be invaded. Competition assumes far less importance in the structure of communities under this concept than it does in more temperate regions.

If the ideas outlined above are accepted, then a central theme of the theory of succession, that of the "climax," becomes difficult of application in boreal regions. One would have to concede that in much of our region no true climax has yet developed in post-glacial time. If we continue to use a concept of climax, it must of necessity be entirely theoretical, or at least highly conditioned.

Polunin seems unwilling to discard the ideas of equilibrium, climax, and seral development, in spite of the troubles he had in defining them. His statement of the problem is worthy of quotation: "Over the exposed limestone plateau which constitutes almost the whole area of the island, the vegetation is so poor as to suggest that hardly any successional advances or even marked changes (except in a few favoured localities) can have taken place since the first colonization after the final ice retreat. Consequently it is to be presumed that the *Dryas* and other main communities now seen, although they may appear to be of almost a pioneer type, will persist at least for a very long time to come. Whether they resemble true climaxes or arrested (sub-climax) stages in an *autogenic main sere,* or merely pioneer or migratory *proseres,* the majority at least of these dwarf and meager plant communities which go to make up the vegetation of Akpatok appear

to be in equilibrium with the present conditions and hence relatively stable" ('35, p. 196).

Cooper has been extraordinarily successful in establishing successional series in the Glacier Bay region of Alaska ('37). Here he has a clean-cut history of recent topographic change following the retreat of the glaciers, with an actual time scale covering a considerable part of it. Furthermore the climate of the region is conducive to relatively heavy forest growth. The analysis of plant communities and seral stages under such conditions becomes almost an exact science.

Although a time scale is not available, quite reasonable successional series can be worked out on flood plain and delta deposits in the northern interior (Raup, '35). Here the change is rapid enough to show some of the earlier stages by which the forests develop. Although Cooper can be fairly confident that the forests being produced on the older surfaces at Glacier Bay are parts of the regional climatic climax, it is doubtful if the northern interior forests can be so classified. The relative simplicity of the latter, both in flora and structure, together with their lack of uniformity, suggest that they have not yet reached the equilibrium of a climatic climax. If Polunin's concept is applied, however, with a somewhat broader interpretation of what constitutes a climax, then these forests might be regarded as actually in equilibrium with conditions in general, and could perhaps be called edaphic subclimaxes.

If recent or current fluctuations of the tree line are valid, then it may well be that an analysis of climax vegetation in the eastern arctic or subarctic would produce quite different results from one in the west. The instability and lack of organization in the Katmai vegetation described by Griggs may be replaced by relative stability in other parts of the arctic. Polunin has postulated a degree of stability at Akpatok Island, and Clarke thinks the forest line is stable in the Thelon region. Cooper has abundant proof of recent development at Glacier Bay, and the present writer finds evidence of unstable conditions in the Mackenzie basin.

Here the matter rests at present, with no well defined bases for generalization. Most of those who have attempted ecological description in boreal America are impressed with the difficulties in the delimitation of associations, the definition of what constitutes equilibrium in a region where associations are so poorly defined,

and the reconstruction of seral development. Added to these troubles is the disturbing failure to adjust the distribution of many species to any climatic values that have yet been set up, and the evidence that northern plant communities may not actually be adjusted to known climatic features. Griggs goes so far as to suggest that some entirely new basis may have to be established before we can form a rational organization of arctic vegetation.

Floristic plant geographers have raised concepts of "conservative" and "aggressive," or "rigid" and "plastic" species; but we do not yet know how significant these categories will be when applied to the organization of plant communities. It has already been proposed that criteria of "species potentiality" will have to be used in this organization before common ground can be found between modern floristic and physiological plant geography. Ecologists have attempted to deal with the situation in the physiological terms of "ecological amplitude," but Cain ('39, p. 161) thinks that this may be conditioned by the age of the species and its relative "capacity for extending its range despite the availability of apparently suitable habitats." In a later paper ('40) he suggests that the biotype depauperation of a species by isolation is more significant than age in determining its capacity to extend its range. The existence of conservatism in species has been sadly lacking of proof, but the recent findings of geneticists (see pp. 61–62*a*) have set it up firmly as at least a good working hypothesis.

It is not impossible that if we are ever to understand the structure and development of boreal plant communities, with their complicated distribution of species and dominance, we will first have to learn where the species lived during the Wisconsin glaciation. Did they survive on large areas where they formed large populations in which their inherent variability could be maintained; or were they parts of limited populations which were depauperated of biotypes and reduced to a "rigid" condition? It is possible that the rôle of a species in the formation of a young boreal community may be determined as much by these inherent limitations as by the interaction of the multitude of external environmental factors.

Two phases of this matter must be borne in mind. First, if such a rating of potentiality for species is set up, we have to deal, not only with extremes, but also with a series of intermediate conditions depending upon the amount of depauperation that has

occurred. Further, some populations that were reduced to uniformity under the influence of isolation during the Wisconsin may subsequently have managed to fuse with others and so to regain their potentialities. Second, the ordinarily accepted species set up by taxonomic criteria may not show the same abilities in migration, establishment, and dominance at different points. Races which show little or no structural peculiarity, isolated from their kind by the ice, may be conservative while larger populations have remained aggressive.

In connection with their studies of speciation, Clausen, Keck and Heisey ('40) make a comment which is significant here, although they do not go into the matter of conservatism vs. aggressiveness: "That modifications and heritable variations have been confused by many botanists is evident in the literature. There has been a paucity of evidence showing to what extent plants may be modified in different types of environment, and how modifications compare with heritable variations. These comparisons have been emphasized . . ., for such a study is fundamental to an understanding of plant interrelationships, and of plants in relation to their environment."

Concepts of conservatism, aggressiveness, and the history of the development of plant communities over long periods of time are to be applied not only in the classic approaches to boreal plant geography, but also in studies based upon the hypothesis of continental drift. It will be noted that the phytogeographic applications of the theory of continental drift are not discussed in the present paper. American students have done very little in this field, but recent papers by Steffen ('37, '38) in Germany serve to outline the current thought and research that touch upon it. E. V. Wulff's paper on an "Introduction to the historical geography of plants" ('32) should also be consulted.

With regard to recent changes of climate and their probable effects upon vegetation boundaries, there are some ecological aspects that need elucidation. As previously stated, Griggs believes that the present tree line in Alaska is not to be correlated directly with contemporary climatic factors, but rather that the forest has lagged behind the change in its favor, and is but slowly advancing to a position of actual adjustment. This idea of "lag" in development is not a new one in phytogeography. Cowles ('01, pp. 79 and 179)

utilized it in his analysis of vegetation in the Chicago region, and Clements ('34) uses it in discussing the "Relict Method in Dynamic Ecology." Rübel ('35; see also Faegri, '37*b*, p. 430) has tried to reduce it to a factoral interpretation in his paper on "The Replaceability of Ecological Factors and the Law of Minimum." Hutchinson ('18) has suggested that the deciduous forests of Ontario have lagged behind climatic changes in their movement northward, and await the further development of soils suitable to them. The present writer has postulated somewhat similar conditions in the central part of the Mackenzie basin ('30), and has suggested also that the forest boundaries in southern New England have shown a tendency to persist after climatic changes have occurred ('37).

The fundamental issue has been stated by Deevey ('39, p. 719), in his discussion of the writer's studies in southern New England: "The concept of widespread and long-continued persistence of forests not adjusted to the prevailing climate logically leads to a negation of ecological theory, and Raup's hypothesis must be construed to include only local areas of relic vegetation." If ecological theory postulates the continued adjustment of vegetation to climate, then Deevey is right; but if the interpretations set forth in the preceding paragraphs are correct, we must postulate conditions wherein vegetation is very often *in process* of adjustment, and actually existing with only partial adjustment. That such conditions need not be local is shown by the large-scale fluctuations of the tree line.

It should be noted in this connection that Hultén arrived at similar conclusions on climatic adjustment in his study of the boreal distribution of species ('37*b*). In fact the idea is inherent in the persistence hypothesis which has been discussed elsewhere in this paper.

Vegetation Mapping. The mapping of vegetation from aerial photographs should yield more useful results than it has to date. Large sections of northern Canada have now been photographed, and maps made showing the general topographic features. The pictures have been made at oblique angles, and from comparatively low altitudes. The writer has demonstrated that vegetation can be mapped in considerable detail from these photographs provided adequate ground studies have been made in selected places (Raup

'35). The maps prepared by the Topographical Survey of Canada, while remarkably accurate, do not have enough detail to serve as bases upon which to put all the vegetational information that can be gained from the photographs. The writer found it necessary, therefore, to construct his own maps from the photographs, with a scale of 4 inches to the mile. A minimum of ground work consists of a series of transects selected so as to cut most of the recognizable types of vegetation. The sites of transects can be located with great accuracy on a photograph and become the "base-lines" or standards from which the whole photograph is analyzed. It is essential to know the time of year in which the pictures were made, since the aspects of the various plant associations change materially during the growing season.

Life-form Classification. Life-form classifications, either on biological or physiognomic bases, have never been made for the boreal American floras except in the arctic. Raunkiaer ('08) published "biological spectra" for many parts of arctic America and Greenland in his classic paper on "The Statistics of Life-forms as a Basis for Biological Plant Geography." His figures were derived from such published lists as were available at the time, and consequently many of them are open to criticism on account of their incompleteness. The only local flora in arctic America that could then be considered well known was that of Ellesmereland (Simmons, '06). It has already been noted that the floras of most of the other regions of the arctic had been only partially collected and studied; and due to the circumstances under which the pioneer work was done the resulting lists very often failed to give a balanced representation of the plant life. If the Raunkiaer system of classification is to be tested thoroughly for boreal America, therefore, much more detailed work is needed in the light of recent floristic studies. This has been well begun by Gelting ('34), and Böcher ('33) in Greenland. So far as the writer is aware, no purely physiognomic system has been applied in our region.

As previously stated, Jones ('36, '38) has made some useful correlations, in western Washington, between the Raunkiaer system and the Merriam life-zone classification. The application of some system of life-form studies to such plant communities as are recognized in boreal America has not yet been tried. The same complicating historical factors that have conditioned the development

of the flora and its arrangement into communities must also be taken into consideration when life-forms are analyzed for their geographic significance. No one has tried to correlate life-form with relative conservatism or aggressiveness.

BIBLIOGRAPHY

ABBE, E. C. 1936. Botanical results of the Grenfell-Forbes northern Labrador expedition, 1931. Rhodora **38**: 102–64.

———. 1938. Phytogeographical observations in northernmost Labrador. Amer. Geo. Soc. Special Publ. 22: 217–34.

———. 1939. The expedition to Hudson Bay of the University of Minnesota. Science **90**: 458–9.

ALBRIGHT, W. D. 1933*a*. Gardens of the Mackenzie. Geog. Rev. **23**: 1–22.

———. 1933*b*. Crop growth in high latitudes. Geog. Rev. **23**: 608–20.

———. 1937. Agriculture and horticulture. *In* Canada's western Northland. pp. 73–81.

ALCOCK, F. J. 1924. Across Gaspé. Geog. Rev. **14**: 208.

———. 1936. Geology of Lake Athabaska region, Saskatchewan. Geol. Surv. Can. Mem. 196.

ALLAN, J. A. 1919. Geology of the Swan Hills in the Lesser Slave Lake district, Alberta. Geol. Surv. Can. Sum. Rep. 1918, C. 7–16.

ANDERSON, E. 1936. The species problems in *Iris*. Ann. Mo. Bot. Gard. **23**: 457–509.

———. 1937. Cytology in its relation to taxonomy. Bot. Rev. **3**: 335–50.

ANDERSON, R. M. 1935. Arctic flora. *In* Canada's eastern arctic, pp. 133–137. Dept. Int. Can. Lands, N. W. T. & Yukon Branch.

———. 1937. Faunas of Canada. Canada Year Book, 1937, pp. 27–52. Can. Dept. Trade & Commerce.

ANTEVS, E. 1925. Retreat of the last ice sheet in eastern Canada. Dept. Mines Can. Geol. Surv. Mem. 146.

———. 1928. The last glaciation. Amer. Geog. Soc. Res. Ser. 17.

———. 1932. Alpine zone of Mt. Washington range.

———. 1934. Climaxes of the last glaciation in North America. Am. Jour. Sci. **28**: 304–11.

———. 1936. Correlations of late quaternary chronologies. Rept. 16th Int. Geol. Cong. Washington, 1933, pp. 213–216.

———. 1938. Climatic variations during the last glaciation in North America. Bull. Am. Meteor. Soc. **19**: 172–6.

BALL, C. R. 1940. Dr. Setchell and Alaska willows. Madroño **5**: 231–2.

BARRELL, J. 1915. Factors in movements of the strand line and their results in the Pleistocene and Post-Pleistocene. Am. Jour. Sci. IV. **40**: 1–22.

BELL, R. 1863. On the superficial geology of the Gaspé Peninsula. Can. Nat. & Geol. **8**: 175.

———. 1884. Observations on the geology, mineralogy, zoology, and botany of the Labrador coast, Hudson's Strait and Bay. Geol. Surv. Can. Rept. Prog. 1882–4, DD.

BISHOP, H. 1930. The Austin collection from the Labrador coast. Rhodora **32**: 59–62.

BLANCHET, G. H. 1924–25. An exploration into the northern plains north and east of Great Slave Lake, including the source of the Coppermine River. Can. Field Nat. **38**: 183–7; **39**: 12–16, 30–34, 52–54.

———. 1926. Great Slave Lake area, Northwest Territories. Dept. Int. Can. N. W. T. & Yukon Branch.

———. 1930. Keewatin and northeastern Mackenzie. Can. Dept. Int., N. W. T. & Yukon Branch.

BÖCHER, T. W. 1933*a*. Phytogeographical studies of the Greenland flora. Meddel. Grønl. **104**(3).

————. 1933*b*. Studies of the vegetation of the east coast of Greenland between Scoresby Sound and Angmagssalik. Meddel. Grønl. **104**(4).

BOWDEN, W. M. 1940. Diploidy, polyploidy, and winter hardiness relationships in the flowering plants. Am. Jour. Bot. **27**: 357–71.

BOWMAN, P. W. 1934. Pollen analysis of Kodiak bogs. Ecology **15**: 97–100.

BROOKS, C. F. 1931. How may one define and study local climates? Compt. Rend. Congrès Int. Geog., Paris.

————, A. J. CONNOR, AND OTHERS. 1936. Climatic maps of North America. Harvard Univ. Press.

BROOKS, S. T. 1936. The land and freshwater mollusca of Newfoundland. Ann. Carnegie Mus. **25**: 83–108.

BRYAN, K. 1928. Glacial climate in non-glaciated regions. Am. Jour. Sci. **16**: 162–4.

CAIN, S. A. 1939. The climax and its complexities. Am. Mid. Nat. **21**: 147–81.

————. 1940. Some observations on the concept of species senescence. Ecology **21**: 213–15.

CAMERON, A. E. 1922. Post-glacial lakes in the Mackenzie River basin, Northwest Territories, Canada. Jour. Geol. **30**: 337–53.

CAMSELL, C. 1916*a*. An exploration of the Tazin and Taltson Rivers, Northwest Territories. Geol. Surv. Can. Mem. 84.

————. 1916*b*. The unexplored areas of continental Canada. Geog. Jour. **48**: 249–57.

————. 1936. Flying through northwestern Canada. Can. Geog. Jour. **12**: 112–22.

CAPPS, S. R. 1932. Glaciation in Alaska. U. S. Geol. Surv. Prof. Paper No. 170.

CAVERHILL, P. Z. 1926. Forest types of British Columbia. *In* Naturalist's Guide to the Americas, pp. 154–5.

CHALMERS, R. 1895. Report on the surface geology of eastern New Brunswick, *etc.* Rept. Geol. Surv. Can. M.

CLARKE, C. H. D. 1940. A biological investigation of the Thelon Game Sanctuary. Nat. Mus. Can. Bull. No. 96, Biol. Ser. 25.

CLAUSEN, J., DAVID D. KECK, AND W. H. HEISEY. 1940. Experimental studies on the nature of species. I. Effect of varied environments on Western North American plants. Carn. Inst. Wash. Publ. 520.

CLEMENTS, F. E. 1934. The relict method in Dynamic ecology. Jour. Ecology **22**: 39–68.

COLEMAN, A. P. 1921. Northeastern part of Labrador and New Quebec. Geol. Surv. Can. Mem. 124.

————. 1922. Physiography and glacial geology of Gaspé, Quebec. Dept. Mines, Can. Bull. 34.

————. 1926. Ice ages, recent and ancient.

CONNOR, A. J. 1937. Climate and weather of the arctic. *In* Canada's Western Northland, pp. 44–7. Can. Dept. of Mines & Res., Lands, Parks and Forests, Branch.

————. 1938. The climates of North America: Second Part, Canada. Handb. Klimat. **2**. (J).

————. 1939. The climate of Manitoba. Publ. Man. Econ. Surv. Board, Winnipeg.

COOPER, W. S. 1923. The recent ecological history of Glacier Bay, Alaska. Ecology **4**: 93–108, 223–46, 355–65.

————. 1931*a*. A third expedition to Glacier Bay, Alaska. Ecology **12**: 61–95.

————. 1931*b*. The seed plants and ferns of the Glacier Bay National Monument, Alaska. Bull. Torr. Bot. Club **57**: 327–38.

———. 1936. The strand and dune flora of the Pacific Coast of North America: a geographic study. *In* Essays in geobotany, pp. 141–87.
———. 1937. The problem of Glacier Bay, Alaska: a study of glacier variations. Geog. Rev. **27**: 37–62.
———. 1939. A fourth expedition to Glacier Bay. Ecology **20**: 130–155.
COWLES, H. C. 1901. The physiographic ecology of Chicago and vicinity; a study of the origin and development of plant societies. Bot. Gaz. **31**: 73–108, 145–82.
CRITCHELL-BULLOCK, J. C. 1930. An expedition to sub-arctic Canada. Can. Field Nat. **44**: 54.
DALY, R. A. 1902. The geology of the northeast coast of Labrador. Bull. Mus. Comp. Zool., Harvard **38**: 203–70.
DAWSON, G. M. 1875. On the superficial geology of the central region of North America. Quart. Jour. Geol. Soc. London **31**: 603–23.
———. 1878. On the superficial geology of British Columbia. Quart. Jour. Geol. Soc. London **34**: 89–123.
———. 1881*a*. Report on an exploration from Port Simpson on the Pacific Coast to Edmonton on the Saskatchewan, embracing a portion of the northern part of British Columbia and the Peace River country, 1879. Geol. Surv. Can. Rept. Prog. 1879–80, B.
———. 1881*b*. Additional observations on the superficial geology of British Columbia and adjacent regions. Quart. Jour. Geol. Soc. London **37**: 272–85.
———. 1888. Report on an exploration in the Yukon District, N. W. T. and adjacent northern portion of British Columbia. Ann. Rept. Geol. Surv. Can. 1887, B.
———. 1891. On the later physiographic geology of the Rocky Mountain region in Canada, with special reference to changes in elevation and to the history of the Glacial Period; being the Presidential Address for the Year. Trans. Roy. Soc. Can. Sec. IV, 1890, 3–74.
DEEVEY, E. S. 1939. Studies on Connecticut lake sediments. Am. Jour. Sci. **237**: 691–724
DENNY, C. S. 1936. Periglacial phenomena in southern Connecticut. Am. Jour. Sci. V. **32**: 322.
———. 1938. Glacial geology of the Black Rock Forest. Black Rock Forest Bull. 8.
———. 1940. Stone-rings on New Hampshire mountains. Am. Jour. Sci. **238**: 432–38.
DEVOLD, J., AND P. F. SCHOLANDER. 1933. Flowering plants and ferns of southeast Greenland. Skrifter om Svalbard og Ishavet 56.
DOBZHANSKY, T. 1937. Genetics and the origin of species.
DOWLING, D. B. 1914. Physical geography of Canada. App. to 13th Rept. Geog. Board Canada, p. 3–15.
DU RIETZ, G. E. 1930. The fundamental units of biological taxonomy. Svensk Bot. Tidsk. **24**: 333–428.
EKBLAW, W. E. 1926. The American arctic archipelago and Ungava and Labrador. *In* Naturalist's Guide to the Americas, pp. 98–111.
ERDTMAN, G. 1931. Worpswede-Wabamum. Abh. Nat. Ver. Bremen **28**: 11–17.
FAEGRI, K. 1937*a*. Some fundamental problems of taxonomy and phylogenetics. Bot. **3**: 400–23.
———. 1937*b*. Some recent publications on phytogeography in Scandinavia. Bot. Rev. **3**: 425–56.
FERNALD, M. L. 1911. A botanical expedition to Newfoundland and southern Labrador. Rhodora **13**: 109–62.
———. 1918. The geographic affinities of the vascular floras of New England, the Maritime Provinces and Newfoundland. Am. Jour. Bot. **5**: 219–47.

———. 1919. Lithological factors limiting the ranges of *Pinus Banksiana* and *Thuja occidentalis*. Rhodora **21**: 41–67.
———. 1921. The Gray Herbarium expedition to Nova Scotia, 1920. Contr. Gray Herb. **63**; or Rhodora **23**: May to Dec.
———. 1924. Isolation and endemism in northeastern America and their relation to the Age and Area Hypothesis. Am. Jour. Bot. **11**: 558–72.
———. 1925. Persistence of plants in unglaciated areas of boreal America. (Mem. Gray Herb. 2); Mem. Am. Acad. 15(3).
———. 1926*a*. *Salix cordifolia* Pursh. *In* Two summers of botanizing in Newfoundland. Rhodora **28**: 181–8.
———. 1926*b*. The antiquity and dispersal of vascular plants. Quart. Rev. Biol. **1**: 212–245.
——— 1929. Some relations of the floras of the northern hemisphere. Proc. Int. Cong. Pl. Sci. **2**: 1487–1507.
———. 1931. Specific segregations and identities in some floras of eastern North America and the Old World. Rhodora **33**: 25–63.
———. 1933. Recent discoveries in the Newfoundland flora. Contr. Gray Herb. **101**. Repr. from Rhod. **35**.
———. 1935. Critical plants of the upper Great Lakes region of Ontario and Michigan. Contr. Gray Herb. **108**; Repr. from Rhodora **37**.
———. 1940. A century of additions to the flora of Virginia, Pt. III, Phytogeographic consideration. Rhodora **42**.
FORBES, E. 1846. On the connection between the distribution of the existing flora and fauna of the British Isles, and the geological changes which have affected their area, *etc*. Mem. Geol. Surv. United Kingdom **1**: 336–432. [For a shorter account see also Ann. & Mag. Nat. Hist. **16**: 126–8, 1845; or British Assoc. Rept., pt. 2, pp. 67–8, 1845.]
GELTING, P. 1934. Studies of the vascular plants of east Greenland between Franz Joseph Fjord and Dove Bay. Meddel, Grønl. **101**(2).
GOLDTHWAIT, R. P. 1939. Glacial geology of the Presidential Range. Thesis, Harv. Univ.
GRAHAM, R. 1934. Notes on the flora of the Peace River. Rep. Prov. Mus. Nat. Hist. Brit. Columbia 1933, pp. 13–24.
GRIGGS, R. F. 1914. Observations on the behavior of some species on the edges of their ranges. Bull. Torr. Bot. Club **41**: 25–49.
———. 1933. The colonization of the Katmai ash, a new and inorganic "soil." Am. Jour. Bot. **20**: 92–113.
———. 1934*a*. The problem of arctic vegetation. Jour. Wash. Acad. Sci. **24**: 153–75.
———. 1934*b*. The edge of the forest in Alaska. Ecology **15**: 80–96.
———. 1936. The vegetation of the Katmai district. Ecology **17**: 380–417.
———. 1937. Timberlines as indicators of climatic trends. Science **85**: 251–5.
———. 1940. The ecology of rare plants. Bull. Torr. Bot. Club **67**: 575–94.
GROH, H. 1937. Peace-Athabaska weeds, a reconnaissance appraisal. Dom. Can. Dept. Agric. Tech. Bull. 7.
GRØNTVED, JOHS. 1936. Vascular plants from arctic North America, etc. Rept. Fifth Thule Exp., 1921–24, **2**: (1).
GÜSSOW, W. C. 1933. Contributions to the knowledge of the flora of northern Manitoba and the Northwest Territories, Dominion of Canada. (Lat. 58°–62°; Long. 95°–100°). Can. Field Nat. **47**: 116–119.
HAGEDOORN, A. L., AND A. C. HAGEDOORN-WORSTHEUVEL LA BRAND. 1921. The relative value of the processes causing evolution.
HAGERUP, O. 1932. Über Polyploidie in Beziehung zu Klima, Ökologie, und Phylogenie; Chromosomenzahlen aus Timbuktu. Hereditas **16**: 19–40.

HALLIDAY, W. E. D. 1937. A forest classification for Canada. Can. Dept. Mines & Res. Forest Serv. Bull. 89.
HARPER, F. 1931. Physiographic and faunal areas in the Athabaska and Great Slave Lakes region. Ecology **12**: 18–32.
HARSHBERGER, J. W. 1911. Phytogeographic survey of North America. Die Vegetatione der Erde **13**.
————. 1928. Unsolved problems in arctic plant geography. *In* Problems of Polar Research, pp. 143–53. Am. Geog. Soc. Spec. Publ. No. 7.
HENRY, J. K. 1915. Flora of southern British Columbia and Vancouver Island.
HENRY, M. G. 1933. Exploring and plant collecting in northern British Columbia. Year Book, Penn. Hort. Soc.
————. 1934. Collecting plants beyond the frontier in northern British Columbia. Nat. Hort. Mag. **13**: 60–75, 162–81, 269–90, 363–83.
HOARE, W. H. B. 1930. Conserving Canada's Musk-oxen. Dept. Int. Can.
HOLM, T. 1922. Contributions to the morphology, synonymy, and geographical distribution of arctic plants. Rep. Canad. Arctic Exped., 1913–18, **5** (B).
HOOKER, J. D. 1862. Outlines of the Distribution of arctic plants. Trans. Linn. Soc. London **23**: 251–348.
————. 1882. On geographical distribution. Brit. Assoc. Rep. 1881: 727–38.
HOOKER, W. J. 1829–1840. Flora Boreali-Americana.
HULTÉN, E. 1937*a*. Flora of the Aleutian Islands.
————. 1937*b*. Outline of the history of arctic and boreal biota during the Quarternary Period.
HUTCHINSON, A. H. 1918. Limiting factors in relation to specific ranges of tolerance of forest trees. Bot. Gaz. **66**: 465–93.
JENNESS, D. 1924. Myths and traditions from northern Alaska, the Mackenzie Delta and Coronation Gulf. Dept. Mines, Can., Rep. Can. Arc. Exp., 1913–18, 8 (A).
————. 1933. The problem of the Eskimo. *In* The American aborigines, their origin and antiquity.
————. 1937. The Indian background of Canadian history. Nat. Mus. Can. Bull. No. 86, Anthrop. Ser. No. 21.
JOHANSEN, FRITZ. 1919. The forest's losing fight in arctic Canada. Can. For. Jour. **15**: 303–5.
————. 1924. General observations on the vegetation. Rep. Canad. Arctic Exped., 1913–18, C.
————. 1933. Description of the country and vegetation at Port Churchill, Manitoba, in arctic Canada, Nyt. Magaz. for Naturvid. **73**: 221–57.
JOHNSON, D. W. 1925. The New England-Acadian shoreline.
JONES, G. N. 1936. A botanical survey of the Olympic Peninsula, Washington. Univ. Wash. Publ. Biol. 5.
————. 1938. The flowering plants and ferns of Mount Rainier. Univ. Wash. Publ. Biol. 7.
KENOYER, LESLIE A. 1940. Notes on plant ecology of northern Ontario. Papers Mich. Acad. Sci. **25**: 67–74.
KINDLE, E. M. 1918. Notes on sedimentation in the Mackenzie basin. Jour. Geol. **26**: 341–360.
KING, RICHARD. 1836. Narrative of a journey to the shores of the Arctic Ocean, *etc.*
KOEPPE, C. E. 1931. The Canadian climate.
LEWIS, F. J., E. S. DOWDING AND E. H. MOSS. 1928. The vegetation of Alberta. II. The swamp, moore and bog forest vegetation of central Alberta. Jour. Ecol. **16**: 19–70.
LIVINGSTON, B. E., AND F. SHREVE. 1921. The distribution of vegetation in the United States as related to climatic conditions. Publ. No. 284, Carnegie Inst. Wash.

LOOMIS, E. 1885. Contributions to meteorology. Am. Jour. Sci. III. **30**: 1–16.
LOW, A. P. 1906. Report on the Dominion Government expedition to Hudson Bay and the arctic islands on board the D. G. S. Neptune, 1903–1904.
MACKENZIE, A. 1801. Voyages from Montreal on the River St. Laurence through the continent of North America to the frozen and Pacific oceans in the years 1789 and 1793, *etc.*
MACOUN, J. M. 1896. List of plants known to occur on the coast and in the interior of the Labrador peninsula. App. 6 Rep. on Expl. Lab. Penin. by A. P. Low. Ann. Rep. Geol. Surv. Can.
———. 1911. Flora and fauna of west coast of Hudson Bay. Summ. Rep. Geol. Surv. Can. 1909–10, pp. 281–3.
———, AND THEO. HOLM. 1921. Vascular plants. Rep. Canad. Arctic Exped., 1913–18. **5** (A).
———, AND M. O. MALTE. 1917. The flora of Canada. Geol. Surv. Can., Mus. Bull. no. **26**: 1–14.
MACOUN, J. 1877. Part of a report on exploration in British Columbia by Alfred R. C. Selwyn, Geol. Surv. Can. Rep. Prog. 1875–6, 110–232.
———. 1883–90. Catalogue of Canadian plants.
MANTON, I. 1932. Introduction to the general cytology of the Cruciferae. Ann. Bot. **46**: 509–554.
MARTIN, L. H. 1925. Geology of the Stonington Region, Connecticut. Conn. Geol. & Nat. Hist. Surv. Bull. 33.
MECKING, L. 1928. The polar regions: a regional geography. *In* The Geography of the Polar Region.s Am. Geog. Soc. Spec. Publ. No. 8.
MERRIAM, C. HART. 1895. Laws of temperature control of the geographical distribution of terrestrial animals and plants. Nat'l Geog. Mag. **6**: 229.
———. 1898. Life zones and crop zones of the United States. U. S. Dept. Agr. Biol. Surv. Bull. 10: 1–79.
MIDDENDORF, A. VON. 1864. Siberische Reise. **4**: 1: 736.
MIDDLETON, W. E. K. 1935. Climate and weather of the eastern arctic. *In* Canada's Eastern Arctic. Can. Dep. Int., Lands, N. W. T. & Yukon Branch, pp. 25–32.
MOSS, E. H. 1932. The vegetation of Alberta. IV. The poplar association and related vegetation of central Alberta. Jour. Ecol. **20**: 380–415.
NATHORST, A. G. 1892. Über den gegenwartigen Standpunkt unserer Kenntniss von dem Vorkommen fossiler Glacialpflanzen. Bih. K. Sv. Vet. Akad. Hdlgr. **17** (5).
NICHOLS, G. E. 1913. The vegetation of Connecticut. I. Torreya **13**: 89–112.
NORDENSKJOLD, O. 1928. Polar nature: a general characterization. *In* The Geography of the Polar Regions. Am. Geog. Soc. Spec. Publ. 8.
NORDHAGEN, R. 1935. Om *Arenaria humifusa, etc.* Bergens Mus. Årbok, Naturv. 1.
NÖRLUND, P. 1924. Buried Norsemen at Herjolfnes. Meddel. Grønl. **67** (1): 1–270.
ODELL, N. E. 1933. The mountains of northern Labrador. Geog. Jour. **82**: 193–210, 315–25.
OSGOOD, W. H. 1926. Alaska. *In* Naturalist's Guide to the Americas, pp. 141–146.
PENNELL, F. W. 1934. *Castilleja* in Alaska and northwestern Canada. Proc. Acad. Nat. Sci. Phila. **86**: 517–40.
POLUNIN, N. 1934. The flora of Akpatok Island, Hudson Strait. Jour. Bot. **72**: 197–204.

———. 1934–35. The vegetation of Akpatok Island. I–II. Jour. Ecol. **22**: 337–95; **23**: 161–209.
———. 1938. The flora of Southampton Island, Hudson Bay. Jour. Bot. **76**: 93–103.
———. 1940. Botany of the Canadian eastern Arctic, Pt. I. Pteridophyta and Spermatophyta. Nat. Mus. Can. Bull. no. 92, Biol. Ser. no. 24.
PORSILD, A. E. 1929. Reindeer grazing in northwest Canada. Dept. Int. Can., N. W. T. & Yukon Branch.
———. 1932. Notes on the occurrence of *Zostera* and *Zannichellia* in Arctic North America. Rhod. **34**: 90–94.
———. 1937*a*. Flora of the Northwest Territories. *In* Canada's Western Northland, pp. 130–141. Can. Dept. Mines & Res., Lands, Parks & Forests Branch.
———. 1937*b*. Edible roots and berries of northern Canada. Can. Dept. Mines & Res., Nat. Mus. of Can.
———. 1938. Earth mounds in unglaciated arctic northwestern America. Geog. Rev. **28**: 46–58.
———. 1938. Flora of Little Diomede Island in Bering Strait. Trans. Roy. Soc. Can. **32**: 21–38.
———. 1939. Contributions to the flora of Alaska. Rhodora **41**: 141–83, 199–254, 262–301.
PORSILD, M. P. 1932. Alien plants and apophytes of Greenland. Meddel. Grønl. **92**: No. 1.
———. 1922. The flora of Greenland: its affinities and probable age and origin. [Abstract.] Torreya **22**: 53–4.
———. 1902. Skildring af Vegetationen paa Disko. Meddel. Grønl. **25**: 1–229.
POTTER, D. 1932. Botanical evidence of a post-pleistocene marine connection between Hudson Bay and the St. Lawrence basin. Rhodora **34**: 69–89, 101–12.
———. 1934. Plants collected in the southern region of James Bay. Rhodora **36**: 274–84.
PREBLE, E. A. 1902. A biological investigation of the Hudson Bay region. U. S. Dept. Agr. Biol. Surv., N. Am. Fauna No. 22.
———. 1908. A biological investigation of the Athabaska-Mackenzie region. U. S. Dept. of Agr. Biol. Surv., N. Am. Fauna No. 27.
———. 1926. The Mackenzie watershed; northern Hudson Bay region, upper Yukon region, and the arctic islands. *In* Naturalist's Guide to the Americas, 115–41.
PULLING, H. E. 1918. Root habit and plant distribution in the far north. Plant World **21**: 223–33.
RAUNKIAER, C. 1934. Livsformernes statistik som grundlag for biologisk plantegeografi. Bot. Tidssk. **29**: 1908. See also The life forms of plants and statistical plant geography, pp. 111–47.
RAUP, H. M. 1930. The distribution and affinities of the vegetation of the Athabasca-Great Slave Lake region. Rhodora **32**: 187–208.
———. 1931. *Salix glauca* L. and its allies in the Athabaska-Great Slave Lake region. Rhodora **33**: 241–4.
———. 1933*a*. Notes on the distribution of white spruce and Banksian pine in northwestern Canada. Jour. Arn. Arb. **14**: 335–44.
———. 1933*b*. Range conditions in the Wood Buffalo Park of western Canada with notes on the history of the wood bison. Spec. Publ. Am. Comm. Int. Wild Life Protect. **1** (2).
———. 1934. Phytogeographic studies in the Peace and upper Liard River regions, Canada, with a catalogue of the vascular plants. Arn. Arb. Contr. **6**.
———. 1935. Botanical investigations in the Wood Buffalo Park. Nat. Mus. Can. Bull. **74**: Biol. Ser. 20.

———. 1936*a*. Pteridophyta and spermatophyta of Southampton Island. Mem. Carn. Mus. **12**: Art. IV, 17–30.

———. 1936*b*. Phytogeographic studies in the Athabaska-Great Slave Lake region, I. Catalogue of the vascular plants. Jour. Arn. Arb. **17**: 180–315.

———. 1937. Recent changes of climate and vegetation in southern New England and adjacent New York. Jour. Arn. Arb. **18**: 79–117.

RICHARDSON, J. 1823. Botanical appendix. Franklin's narrative of a journey to the shores of the polar Sea, *etc*.

———. 1851. Arctic searching expedition: a journal of a boat voyage through Rupert's Land and the arctic sea in search of the discovery ships under command of Sir John Franklin.

ROSS, B. R. 1862. An account of the botanical and mineral products used by the Chippewayan Indians. Can. Nat. **7**: 133.

RÜBEL, E. 1935. The replaceability of ecological factors and the law of minimum. Ecology **16**: 336–41.

RUTHERFORD, R. L. 1930. Geology and water resources in parts of the Peace River and Grande Prairie districts, Alberta. Rep. 21, Nat'l Res. Council Alta. Geol. Surv. Div., Edmonton.

RYDBERG, P. A. 1913–1921. Phytogeographical notes on the Rocky Mountain region, Parts 1–10. Bull. Torr. Bot. Club **40–48**.

———. 1922. Flora of the Rocky Mountains and adjacent plains.

———. 1932. Flora of the prairies and plains of central North America.

SCAMMAN, E. 1940. A list of plants from interior Alaska. Rhodora **42**: 309–43.

SCHARFF, R. F. 1912. Distribution and origin of life in America.

SCHNEIDER, C. 1921. Notes on American willows. XII. Jour. Arn. Arb. **3**: 61–125.

SCOTT, I. D. 1926. Ice-push on lake shores. Papers Mich. Acad. Sci. **7**: 107–23.

SEIDENFADEN, G. 1931. Moving soil and vegetation in East Greenland. Meddel. Grønl. **87** (2).

SETON, E. T. 1911. The arctic prairies, a canoe journey of 2000 miles in search of the caribou.

SHAW, SIR N. 1936. Manual of meteorology **2**: 365–8.

SIMMONS, H. G. 1906. The vascular plants in the flora of Ellesmereland. Rep. 2nd Norwegian Arc. Exp. in the "Fram," 1898–1902, No. 2.

———. 1913. A survey of the phytogeography of the arctic American archipelago. Lunds Univ. Arssk. N. F. Afd. 2, **9**(19).

SMITH, P. S. 1939. Areal geology of Alaska. U. S. Geol. Surv. Prof. Paper No. **192**.

———, AND J. B. MERTIE. 1930. Geology and mineral resources of northwestern Alaska. U. S. Geol. Surv. Bull. **815**.

SØRENSEN, T. 1933. The vascular plants of east Greenland from 71° 00′ to 73° 30′ N. Lat. Meddel. Grønl. **101**(3).

———. 1935. Bodenformen und Pflanzendecke in Nordostgrønland. Meddel. Grønl. **93**(4): 1–67.

STEBBINS, G. L. 1940. The significance of polyploidy in plant evolution. Am. Nat. **74**: 54–56.

STEFFEN, H. 1937–38. Gedanken zur Entwicklungsgeschichte der arktischen Flora, I–IV. Bot. Centralbl. **56B**: 409–27; **57B**: 367–430; **58B**: 141–202.

———. 1938. Die Entwicklung der arktischen Flora auf der Grundlage der Wegener'schen Theorie. Natur u. Volk. **68**(4): 184–194.

STOCKWELL, C. H., AND D. F. KIDD. 1932. Metalliferous mineral possibilities on the mainland part of the Northwest Territories. Geol. Surv. Can. Sum. Rep., 1931, C., 70–85.

STÖRMER, PER. 1933. Plants collected by Frits Johansen in 1929, at Hudson Bay Railway and Port Churchill in arctic Canada. Nyt. Magaz. for Naturvid. **73**: 259–72.
STUPART, R. F. 1928. The influence of arctic meteorology on the climate of Canada especially. *In* Problems of Polar Research. Am. Geog. Soc. Spec. Publ. 7.
THOMPSON, D. 1916. Narrative of his explorations in western America, 1784–1812. [Edited by J. B. Tyrrell.]
Topographical Survey of Canada. National Topographic Series, Sheet Nos. 74 M, 74 N, 74 O, 74 P.
TURESSON, G. 1922. The genotypical response of the plant species to the habitat. Hereditas **3**: 211–350.
TYRRELL, J. B., ASSISTED BY D. B. DOWLING. 1896. Report on the country between Athabasca Lake and Churchill River, *etc.* Geol. Surv. Can. Ann. Rep. **8** (D).
——————. 1897. Report on the Doobaunt, Kazan and Ferguson Rivers, and the northwest coast of Hudson Bay, *etc.* Geol. Surv. Can. **9** (F).
——————. 1898. The glaciation of north-central Canada. Jour. Geol. **6**: 147–60.
——————. 1910*a*. Ice on Canadian lakes. Trans. Can. Inst. **9**: 13–21.
——————. 1910*b*. Changes of climate in northwestern Canada since the glacial period. *In* Die Veränderungen des Klimas seit dem Maximum der letzten Eiszeit. 389–91. 11th Int. Geol. Congress, Stockholm.
TYRRELL, J. W. 1898. Across the sub-arctics of Canada.
——————. 1901. Report on an exploratory survey between Great Slave Lake and Hudson Bay, *etc.* Dept. Int. Can., Pt. 3, 98–131.
VICTORIN, FR. MARIE-. 1935. Flore Laurentienne.
——————. 1938. Phytogeographical problems of eastern Canada. Contr. Lab. Bot. Univ. Montreal, No. 30: Repr. from Am. Mid. Nat. **19**: 489–558.
WARD, R. DEC., AND C. F. BROOKS. 1936. The climates of North America: First Part, Mexico, United States, Alaska. Handb. Klimat. **2** (J).
WARMING, EUG. 1888. Om Grønlands Vegetation. Meddel. Grønl. **12**: 1–245.
——————. 1888. Über Grønlands Vegetation. Engler Bot. Jahrbücher **10**(4): 364–409.
WEAVER, J. E., AND F. E. CLEMENTS. 1938. Plant ecology.
WETMORE, R. H. 1923. Plants of Labrador. Rhodora **25**: 4–12.
WHITFORD, H. N., AND R. D. CRAIG. 1918. Forests of British Columbia. Comm. Conserv., Ottawa.
WOODWORTH, R. H. 1927. Interesting plants of northern Labrador. Rhodora **29**: 54–57.
WRIGHT, S. 1931. Evolution in mendelian populations. Genetics **16**: 97–159.
——————. 1932. The rôles of mutation, inbreeding, crossbreeding, and selection in evolution. Proc. 6th Int. Cong. Genet., Ithaca. **1**: 356–66.
WULFF, E. V. 1932. Introduction to the historical geography of plants. Bull. Applied Bot. of Genetics and Pl. Breeding, Leningrad, Suppl. 52. In Russian, with summary in English.
WYNNE-EDWARDS, V. C. 1937. Isolated arctic-alpine floras in eastern North America: a discussion of their glacial and recent history. Trans. Roy. Soc. Can. III (5), **31**: pp. 1–26.
——————. 1939. Some factors in the isolation of rare alpine plants. Trans. Roy. Soc. Can. III **33**(5): 35–42.

THE BOTANICAL REVIEW

VOL. 7 MAY, 1941 No. 5

PLASMODESMATA

A. D. J. MEEUSE
The University, Leiden

HISTORICAL DATA

Plasmodesmata, the connecting threads of protoplasm which pass through the walls of cells, were first described by Tangl (70) in 1879 though several botanists had previously seen these structures —Hofmeister, Dippel, Goroschankin—without, however, recognizing the importance of their observations. Soon after publication of Tangl's paper, a number of investigators tried to show the general presence of plasmodesmata in the plant kingdom (10–14, 23, 30–33, 60, 61, 77, 78) as well as in various tissues of single species (19, 37, 40). At the same time the methods for demonstrating their presence were developed, but, as many of them included too drastic swelling of the wall, several investigators came to unreliable results. Criticisms were given by Meyer (47) and Strasburger (66), and an excellent review of the literature up to 1901 is included in the latter reference. After Strasburger's treatment, the study was rather neglected. In 1920 and 1922, respectively, Meyer (52) and Lundegårdh (41) published reviews of the subject, and their statements have been generally accepted in several text books (67, 80, 63, 39). In 1930 Jungers (26) cast doubt upon the alleged protoplasmic nature of plasmodesmata, but later investigations brought forth new evidence that plasmodesmata are protoplasmic in nature (42), so that the old views held by Tangl are still valid.

METHODS FOR DEMONSTRATING THE PRESENCE OF PLASMODESMATA

As a rule, plasmodesmata may be demonstrated only by special technique. Though many methods have been worked out, only a few have found general application. Most of them are variations of the same treatment, and involve soaking in iodine reagents after or without previous fixation, followed by swelling of the wall in zinc chloride solution or various concentrations of sulphuric acid,

and then by staining in some violet stain (4, 54, 67, 80). Sometimes, however, it is possible to see the plasmodesmata without special treatment or after simple staining. The methods may be distinguished as follows:

1. Direct observation, possible only in exceptional cases, as in living colonies of *Volvox aureus* (49) and in some endosperms or cotyledons, *e.g.*, of *Strychnos nux-vomica* (70), *Diospyros* (59), *Aesculus Hippocastanum* (44) and probably *Phytelephas macrocarpa* and *Raphia taedigera* (79);

2. A single stain, as iodine (50, 70), haematoxylin (4, 50, 59) or anilines (4, 50, 54); as a rule, applicable only to endosperm;

3. Special methods. According to Mühldorf (54), most of these are variations of the old reaction of iodine reagents on cellulose followed by sulphuric acid or zinc chloride solution. If a stain is applied after swelling, it generally gives a black precipitation with iodine which is absorbed more intensely by the protoplasmic plasmodesmata than by the wall, thus rendering a marked differential staining. A full account is given by Mühldorf (54). Meyer's "pyoktanin" method, originally developed by Gardiner (10) with impure stains or others of unknown origin, is used in various modifications, namely, Kuhla's (37), Strasburger's (66), Junger's (26) and Craft's (5). As a rule, it is supposed to be the best of all and even claimed to yield a specific differential staining (26, 40). According to Mühldorf (54), however, the reaction with iodine and sulphuric acid gives the most reliable results, for the pyoktanin method often produces precipitations in the walls simulating plasmodesmata. Gardiner's favorite methods (13) are very intricate and were used only by himself and by Hill (19, 20, 22). Mühldorf considers them very unreliable. Silver impregnation has been employed (16, 55, 74) and may give excellent results in some cases (54), as calloused sieve-tubes, but with Pfeiffer-Wellheim's modification precipitations are likely to occur. Mühldorf (54), using endosperms of *Strychnos nux-vomica* and of palms, obtained excellent results by hardening the plasmodesmata for weeks in formalin followed by staining, after swelling in sulphuric acid with pyoktanin. He also was able to produce positive tests on "oxydase" in the plasmodesmata by applying several reagents, especially Schulz and Gräff's "Nadi reagent." We must agree with Mühldorf's statement that as many methods as possible should be applied to a single sub-

ject in order to avoid misinterpretations caused by precipitations and other artefacts.

NOMENCLATURE

Tangl (70) referred to plasmodesmata as "offene Communicationen" between the protoplasts, but the name was not adopted and others were proposed, viz.: connecting threads (12–14, 19) Plasmaverbindungen (4, 30, 32–37, 48–50, 56), and Zell- or Plasmabrücken (52, 54). Strasburger's term of Plasmodesmen (66) found rather general employment (26, 28, 29, 31, 39, 40, 55, 58, 62, 63, 67, 74, 77, 78, 80), but there has been much variation in the form of the term: plasmodesmen, plasmodesma and plasmodesmata (plural sense). We propose to follow Livingston (40) and to employ "plasmodesma" in the singular and "plasmodesmata" in the plural sense. Plasmodesmata and protoplasmic connections are not equivalent. The conspicuous rods of protoplasm traversing the sieve-plates of active sieve-tubes are protoplasmic connections, but not plasmodesmata. Meyer (52) has discussed this problem and separates the true plasmodesmata, his "Zellbrücken," from the other, much wider, protoplasmic connections, his "Fusionsbrücken." Zellbrücken are very fine strands, not possessing microscopically visible protoplasmic inclusions and not showing any convection streaming. Fusionsbrücken are wider and may allow visible particles of the protoplasts to migrate from cell to cell. It must be borne in mind, however, that sometimes plasmodesmata may be converted into Zellbrücken, and *vice versa,* in sieve-plates (20–22, 54) and in the thallus threads of *Codium* (38).

In conclusion, we may define plasmodesmata as living threads of protoplasm connecting adjacent protoplasts through non-living substances separating the protoplasts (cell walls; mucilaginous intercellular substances in *Volvox*-colonies), thus establishing a correlated entity of interconnecting protoplasts. They are never so wide as to permit real fusion of protoplasts or migration of protoplasmic inclusions.

DISTRIBUTION OF PLASMODESMATA

Soon after Tangl's first publication, the great importance of plasmodesmata in connection with translocation was realized, and attempts were made to show their general presence in the plant king-

dom and in all living tissues of any individual. Many investigators have been misled by artefacts, so that much of their data is unreliable and has been discredited by Meyer (47) and Mühldorf (54). The latter author concluded that plasmodesmata are not present in bacteria, Cyanophyceae, Rhodophyta, fungi and lichens, in which they had been reported by several other authors (*e.g.*, 48), and, as a rule, are wanting in the Chlorophyta, except *Volvox*, *Chaetopeltis* (32, 35, 47) and *Codium* (38). In the Phaeophyta with well developed sieve hyphae, Sykes (69) observed conspicuous protoplasmic connections closely resembling the slime strings in Anthophyta which occasionally may be narrowed by a callous-like substance; these protoplasmic connections may be thus converted into real plasmodesmata, as in the sieve-plates of Anthophyta (54). In the less specialized Phaeophyta true plasmodesmata are apparently absent.

In all higher plants plasmodesmata are present between all living cells, and have been definitely established in mosses (31, 33, 54, 66), Hepaticae (54), Pteridophyta (54, 57), and Anthophyta (10–12, 19, 31, 35, 37, 40, 60, 66, 77, 78), including gymnosperms (15, 19, 65, 66). They occur even in meristematic tissues (29, 41, 43, 52), but not in the youngest walls formed immediately after cell division (54).

The presence of plasmodesmata in these plants forms another difference between the "true" tissues of Bryophyta, Pteridophyta and Anthophyta and the "false" tissues in the thalli of lower cryptogams, the algae and fungi, the former being a real entity in every respect, the latter an aggregation of separate elements (hyphae or other thallus filaments).

Plasmodesmata are also present between animal cells (68), and special interest attaches to those which may be observed in living organisms, as *Volvox* (49), and in walls separating cells in graft hybrids (51).

CONFUSION OF PLASMODESMATA WITH OTHER STRUCTURES OR WITH ARTEFACTS

Other cell wall structures have often been confused with plasmodesmata and, like artefacts, have misled many investigators. Therefore, many statements are doubtful and have been contested (42, 47, 54) on good grounds, rendering many papers published before

1937 worthless. Even after Mühldorf's publication, other cell wall structures were erroneously taken for plasmodesmata (42, 62). It is necessary, therefore, to call attention to them:

A. Structural variations of the wall, sometimes made more visible by complicated techniques:

a. Very narrow pit cavities (23, 30, 47, 54, 66).

b. Dermatosome-like granules, caused by swelling agents, often in linear arrangements and showing beaded or granular appearance (26).

c. Variations of cellulose content in different parts of the wall, often very conspicuous after applying iodine and a swelling reagent (pyoktanin method). Of special interest are the "striations" (45) which are certainly not protoplasmic (46); they have led many investigators to the wrong conclusion that plasmodesmata occur in the outer wall of the epidermis or seed coat (12, 26, 42, 54, 62, 66).

B. Structures developed incidentally:

d. Lines produced by the knife during sectioning, especially in hard or "bony" endosperm (*Phytelephas, Strychnos, Lupinus*).

e. Granular precipitation in the wall (Meyer's pyoktanin, Pfeiffer-Wellheim's silver impregnation); sometimes the resemblance is striking, especially when the granules are linearly arranged.

f. Stained granules in other protoplasmic parts of the cell, or black granular precipitations in the protoplast near the wall; when not properly focused they may appear as lines.

g. Dust particles, wax granules or bacteria.

h. Iodine crystals.

i. Threads of protoplasm, present after plasmolysis, between the wall and the contracted protoplast.

COMPOSITION AND MORPHOLOGY

Observation of living, unstained plasmodesmata (*Volvox,* 49, 52) indicate that they are tenuous, terete, hyaline, thread-like structures. Studies on endosperms and *Volvox,* when stained without swelling, show that plasmodesmata do not possess an exceptionally fine structure and that fixation and swelling may change their appearance, so that in preparations granular or beaded plasmodesmata are often noted. Sometimes a plasmodesma is replaced by a row of

separate granules which are distinguished only with difficulty from other rows of granules. A central nodule is often observed in a plasmodesma, but this is also an artefact caused by the different rate of swelling of the cell wall proper and of the middle lamella (34, 35, 52, 66).

Meyer (49), on account of the behavior of the plasmodesmata of *Volvox* towards fixing agents, came to the conclusion that they behave in the same way as protoplasm. Tangl (70) found that plasmodesmata stain with iodine in the same way as the outer layer of the protoplasts, and that they resemble this outer hyaline layer very much.

The general assumption that plasmodesmata are of protoplasmic nature is difficult to prove by direct evidence. Mühldorf (53) was able to demonstrate that the walls of seeds of the Umbelliferae, very rich in plasmodesmata, show marked protein reactions which are less conspicuous after treatment with proteolytic enzymes. Sometimes the plasmodesmata blacken with osmic acid after treatment with proteolytic enzymes (54), which is an indication of lipoids (lipoproteins). Positive "oxydase"-tests have frequently been observed (54). As a rule, plasmodesmata are never branched except in *Volvox,* and all other reports to the contrary (26, 66) have been caused by misinterpretations (42, 54). Plasmodesmata generally run straight from cell to cell, and only in the neighborhood of intercellular spaces or pit membranes of narrow pits are the outer plasmodesmata curved, resembling spindle threads of mitotic figures.

Plasmodesmata occur either in groups or equally distributed through the wall. Kohl (34) assumed a dimorphism among them and distinguished "aggregierte und solitäre Plasmodesmen." This distinction, however, has no special significance, for otherwise the two types are not different, and the distinction rests only on the fact that plasmodesmata are often concentrated in pit membranes. Every pit membrane, as a rule, contains a group of them and each group represents a group of "aggregated" plasmodesmata.

Meyer's (52) nomenclature deserves greater recognition, for he distinguishes "Wand- und Tüpfelbrücken" (wall and pit plasmodesmata). These two types may occur in the same cell wall, as in the endosperm of *Phytelephas macrocarpa.*

The presence of pits is often explained by stating that plasmodesmata are confined to certain parts of the meristematic cell walls

and that in these places secondary wall layers are not deposited, so that the primary wall remains unthickened at those points, and becomes a pit membrane. The places where these groups of plasmodesmata are found, are very conspicuous in the cambium (29, 43) and are called "primary pit fields." They are noted in the walls of meristems passing gradually into parenchyma and in the finest meshes of the reticulate wall structure described and figured by Baranetzki (2) and Tupper-Carey and Priestley (76).

TRUE PROTOPLASMIC NATURE OF PLASMODESMATA

Jungers (26) detected the protoplasmic nature of plasmodesmata, but his findings have been criticized (40, 54). A full account of this subject is given elsewhere (42); we give therefore only a few arguments pleading evidence for the protoplasmic nature of plasmodesmata which is now definitely established:

a. Plasmodesmata are present in walls of living cells but not in dead tissues;

b. Direct continuity of protoplasts and plasmodesmata has often been observed;

c. Plasmodesmata may be converted into "slime strings" of sieve-tubes, and *vice versa;*

d. Plasmodesmata and protoplasts show the same affinity for stains, especially iodine;

e. There is translocation of viruses from cell to cell;

f. Positive tests on "oxydases" also provide evidence.

DEVELOPMENT OF PLASMODESMATA

Kienitz-Gerloff (30) and Strasburger (66) have disposed of Russow's (60) hypothesis that plasmodesmata are the remains of the spindle threads of mitoses, an idea also held by Gardiner (14). Russow's notion was based on the superficial resemblance of groups of plasmodesmata to a mitotic spindle, which resemblance had been noticed before by Tangl. Strasburger remarks quite correctly that some walls do not develop as a result of mitosis, but by the growth of existing walls (periclinal walls of apical meristems, radial walls in the cambium) without showing appreciable differences in the number of plasmodesmata as compared with other walls of the same cells arising from a cell plate or phragmoplast (37) involved in cell divisions. Other explanations of plasmodesmata have not as yet been proposed.

The fact that plasmodesmata have not been demonstrated in very young walls immediately after cell division (54, 66), but that they suddenly appear in older stages, may be explained in three different ways:

a. Plasmodesmata may be present, but our technique has not been sufficiently developed to detect them. This failure is often ascribed to the fact that very young walls do not swell.

b. Plasmodesmata may actually be lacking in the youngest walls but formed later (54, 66).

c. Young cell walls are penetrated by protoplasm and the protoplasts are thus in communication, but later the protoplasm may retract from the walls except in certain places where it then constitutes the plasmodesmata.

The first of these explanations is the least likely, since so many different techniques have failed to detect them.

The second explanation assumes that the function of plasmodesmata—translocation—is performed otherwise when the latter are not present. Mühldorf thought that substances pass directly through the thin walls. Strasburger was of the opinion that plasmodesmata are formed by intrusions of the protoplasts of adjacent cells, penetrating the wall from both sides and meeting in the middle. This hypothesis can not be supported, however, and it has been shown, on the contrary, by Strasburger himself, that plasmodesmata after having been ruptured by plasmolysis are not re-established. It is also highly improbable that at the same time the wall is locally digested at some points by the intrusions, and thickened by the same protoplasts at other points. It seems impossible to the author of this review that the protoplast, a colloid, should penetrate the wall, a gel, for colloidal substances probably do not penetrate gels. Intermediate stages to be expected in the development of plasmodesmata, *e.g.*, two protoplasmic intrusions not yet meeting in the middle and still separated by a part of the wall, have never been observed.

The third explanation seems to be the most acceptable and does not conflict with the sudden appearance of plasmodesmata and the continuity of the protoplasts through the walls. In addition, the fine structure of meristematic walls (43, 76) indicates that certain places remain unthickened, forming meshes in the net of cell wall thickenings (2, 43, 58). Accordingly, the presence of plasmodesmata may cause the formation of pits.

In certain kinds of growing cell walls the number of plasmodesmata increases along with growth of the cell, suggesting a formation of "secondary" plasmodesmata. This increase, most probably and for the reasons already discussed, is not brought about by protoplasmic intrusions into the wall meeting in the middle. We suggest, on the contrary, that the increase comes about by a splitting of the first plasmodesmata, which is not at all unlikely, for since the surface area of the wall continuously increases in growing cells, the cross sectional area of the channels occupied by the plasmodesmata also increases. Cellulose is not deposited in these channels, as in other parts of the wall, and the diameter of the plasmodesmata is thus permitted to increase. After a while the plasmodesma becomes so wide that cellulose deposit occurs in it, and it is thus divided into two or more daughter plasmodesmata (58). The appearance of visible plasmodesmata may be caused also by the submicroscopic pores in the very young walls, filled with protoplasm, increasing in cross section to such a degree that they become microscopically visible and in themselves appear as plasmodesmata.

When cells establish secondary contacts with other cells, as in cambium, in the embryo of *Euphorbia* (43) and in graft hybrids (3, 24, 51, 52, 66), plasmodesmata may form in the walls which arise from these secondary contacts. These are often supposed to be "secondary" plasmodesmata, but in meristematic tissues this need not be the case. The walls of meristematic cells establishing secondary contacts are penetrated by protoplasm, and the adjoining protoplasts, since the middle lamellae for the most part are extremely thin (29, 43), coalesce into one another. Plasmodesmata may then develop in the same way as in other meristematic cell walls. This meeting of protoplasts is possible, for the meristematic walls are not rich in cellulose (43), and therefore are much more porous than older walls. In less meristematic tissues the walls are no longer penetrated by protoplasm and plasmodesmata can not develop. This is in agreement with the fact that walls developed from secondary contacts of growing young vessels and wood fibers frequently do not possess plasmodesmata and consequently do not form pits, as may often be observed (58).

Plasmodesmata, therefore, always arise in the same way, namely, as connections of adjacent protoplasts, being microscopically visible,

or submicroscopic in very young meristematic walls. This is true also when there are secondary contacts.

THE RÔLE OF PLASMODESMATA

Apart from the function of plasmodesmata as channels of translocation, there are several other processes in which they are involved. It has often been assumed that growing cells establish secondary contacts by "sliding growth" (36) which involves a mutual slipping of cells along one another with resulting rupture of plasmodesmata which must subsequently be reformed. This view was held by Krabbe (36) and Jost (25). Complete loss of all plasmodesmata at any one time seems highly improbable, for it would completely isolate the protoplasts. Therefore, Krabbe's concept of sliding growth has been replaced by Priestley's idea of "symplastic growth" (58) which assumes a readjustment of all cell walls as a "common framework" (58) without slip and without any rupture of plasmodesmata, and by Sinnott and Bloch's concept of "intrusive growth" which assumes a local apical growth and local rupture of plasmodesmata. These concepts have been reconsidered recently (43) and it appears that Priestley's concept generally is valid, but that in certain cases new contacts of cells are established in which plasmodesmata may develop if the cell is still meristematic. There is no direct evidence in support of the contention (63, 66) that protoplasmic connections may reform after being destroyed. The presence of plasmodesmata, therefore, may be used as an argument against rupture and consequently against "sliding growth" and "intrusive growth" (43, 58).

The conspicuous protoplasmic connections in sieve-plates seem to develop from plasmodesmata (20–22, 54, 73) and *vice versa* (54). An analogous process is found in some germinating seeds, in which the enormous quantities of storage food in the walls of the endosperms are mobilized by enzymatic breakdown. Disintegration of the walls proceeds along the plasmodesmata (12, 54). It is often assumed that the presence of a great number of plasmodesmata in the walls of endosperms may be an important factor in the mobilization of storage food by increasing the surface of the protoplasts. This is given as an explanation of the presence of two types of plasmodesmata (pit plasmodesmata and wall plasmodesmata) in the endosperm of *Phytelephas macrocarpa* (66), the pit plasmodes-

mata acting as true protoplasmic connections and having their normal functions, the wall plasmodesmata acting only in germinating seeds when accelerating the enzymatic breakdown by increasing the exposed surface. The importance of plasmodesmata in translocation of solids has been discussed elsewhere (9, 40, 54, 66).

No experiments have as yet been published demonstrating actual translocation of solids from cell to cell through plasmodesmata. The idea of this rôle is founded only on indirect evidence, as the translocation of virus from cell to cell (40) and the progress of enzymatic breakdown along the plasmodesmata. We may expect to know more about this aspect when more is known concerning the translocation of materials in sieve-tubes. Conduction of stimuli has been regarded as a second function (17), but direct evidence for this, too, is wanting. We must conclude, therefore, that the true functions of plasmodesmata have not been definitely determined.

Bibliography

1. Bálint, S. Botanische mikrotechnische Notizen. Zeits. Wiss. Mikr. **27**: 243–247. 1910.
2. Baranetzki, J. Epaississement des parois des éléments parenchymateux. Ann. Sci. Nat. Bot. VII. **4**: 135–301. 1886.
3. Bruder, J. Studien an *Laburnum Adami.* Zeits. Induk. Abst. Vererb. **5**: 209–284. 1911.
4. Chamberlain, C. J. Methods in plant histology. 5th Ed. 145–148. 1932.
5. Crafts, A. S. A technic for demonstrating plasmodesma. Stain Tech. **6**: 127–128. 1931.
6. ———. The relation between structure and function of the phloem. Am. Jour. Bot. **26**: 172–177. 1939.
7. ———. The protoplasmic properties of sieve-tubes. Protoplasma **33**: 389–398. 1939.
8. Dippel, L. Das Mikroskop und seine Anwendung. II. 1898.
9. Esau, K. Some anatomical aspects of plant virus disease problems. Bot. Rev. **4**: 548–579. 1939.
10. Gardiner, W. The continuity of the protoplasm through the walls of vegetable cells. Phil. Trans. Roy. Soc. London, Part 3, **174**: 817–863. 1883.
11. ———. On the continuity of the protoplasm through the walls of vegetable cells. Arb. Bot. Inst. Würzburg **3**: 52–87. 1883.
12. ———. The histology of the cell wall, with special reference to the mode of connection of cells. Proc. Roy. Soc. London **62**: 100–112. 1897.
13. ———. Methods for the demonstration of "connecting threads" in the cell wall. Proc. Cambridge Phil. Soc. **9**: 504–508. 1898.
14. ———. The genesis and development of the walls and connecting threads of the plant. Proc. Roy. Soc. London **66**: 186–188. 1900.
15. Goroschankin, J. Zur Kenntnis der Corpuscula bei den Gymnospermen. Bot. Zeit. **41**: 825–831. 1883.
16. Günther, O. Beiträge zur Kenntnis der Entwicklung des Getreide-endosperms und seines Verhalten bei der Keimung. Bot. Arch. **18**: 209–319. 1927.
17. Haberlandt, G. Physiologische Pflanzenanatomie. 6th Ed. 594. 1924.

18. HECHT, K. Studien über den Vorgang der Plasmolyse. Beih. Biol. Pflanzen 11: 137–192. 1912.
19. HILL, A. W. The distribution and character of "connecting threads" in the tissues of *Pinus sylvestris* and other allied species. Phil. Trans. Roy. Soc. London B194: 83–125. 1901.
20. ———. The histology of the sieve-tubes of *Pinus*. Ann. Bot. 15: 575–611. 1901.
21. ———. Notes on the histology of the sieve-tubes of certain angiosperms. Ann. Bot. 17: 265–267. 1903.
22. ———. The histology of the sieve-tubes of angiosperms. Ann. Bot. 22: 245–290. 1908.
23. HILLHOUSE, W. Einige Beobachtungen über den interzellularen Zusammenhang von Protoplasten. Bot. Centralbl. 14: 89–94, 121–124. 1883.
24. HUME, M. On the presence of connecting threads in graft hybrids. New Phytol. 12: 216–225. 1913.
25. JOST, L. Ueber einige Eigentümlichkeiten des Cambiums der Bäume. Bot. Zeit. 59: 1–24. 1901.
26. JUNGERS, V. Recherches sur les plasmodesmes chez les végétaux. La Cellule 40: 7–82. 1930.
27. ———. Figures caryocinétiques et cloisonnement du protoplasme de l'endosperme d'*Iris*. La Cellule 40: 293–354. 1931.
28. ———. Recherches sur les plasmodesmes chez les végétaux. II. Les synapses des algues rouges. La Cellule 42: 7–28. 1933.
29. KERR, I., AND BAILEY, I. W. The cambium and its derivative tissues. X. Structure, optical properties and chemical composition of the so-called middle lamella. Jour. Arn. Arb. 15: 327–349. 1934.
30. KIENITZ-GERLOFF, F. Die Protoplasmaverbindungen zwischen benachbarten Gewebselementen in der Pflanze. Bot. Zeit. 49: 1–10, 17–26, 32–46, 48–60, 64–74. 1891.
31. ———. Neue Studien über Plasmodesmen. Ber. Deut. Bot. Ges. 20: 93–117. 1902.
32. KOHL, F. G. Protoplasmaverbindungen bei den Algen. Ber. Deut. Bot. Ges. 9: 9–17. 1891.
33. ———. Die Protoplasmaverbindungen der Spaltöffnungsschliesszellen und der Moosblattzellen. Bot. Centralbl. 72: 257–265. 1897.
34. ———. Dimorphismus der Plasmaverbindungen. Ber. Deut. Bot. Ges. 18: 364–372. 1900.
35. ———. Beiträge zur Kenntnis der Plasmaverbindungen in den Pflanzen. Beih. Bot. Centralbl. 12: 343–350. 1902.
36. KRABBE, G. Das gleitende Wachstum bei der Gewebebildung der Gefässpflanzen. 1886.
37. KUHLA, F. Die Plasmaverbindungen bei *Viscum album*. Bot. Zeit. 58: 29–58. 1900.
38. KÜSTER, E. Die Plasmodesmen von *Codium*. Protoplasma 19: 335–349. 1933.
39. ———. Die Pflanzenzelle. 1935.
40. LIVINGSTON, L. G. The nature and distribution of plasmodesmata in the tobacco plant. Am. Jour. Bot. 32: 75–87. 1933.
41. LUNDEGÅRDH, H. Zelle und Zytoplasma in: Linsbauers Handbuch der Pflanzenanatomie, 1. Abt. 1 Teil: Zytologie. 1922.
42. MEEUSE, A. D. J. On the nature of plasmodesmata. Protoplasma 35: 143–151. 1941.
43. ———. Rec. Trav. Bot. Néerl. 1941.
44. ———. (Unpublished work.)
45. MEEUSE, B. J. D. Some observations on special structures in the cell walls of plants. Proc. Kon. Akad. Wet. Amsterdam 41: 965–975. 1938.

46. ————. (Unpublished work.)
47. MEYER, A. Das Irrtümliche der Angaben über das Vorkommen dicker Plasmaverbindungen zewischen den Parenchymzellen einiger Filicineen und Angiospermen. Ber. Deut. Bot. Ges. **14**: 154–158. 1896.
48. ————. Das Vorkommen von Plasmaverbindungen bei den Pilzen. Ber. Deut. Ges. **14**: 280–281. 1896.
49. ————. Die Plasmaverbindingungen und die Membran von *Volvox globator, aureus* and *tertius,* mit Rücksicht auf die thierischen Zellen. Bot. Zeit. **54**: 187–217. 1896.
50. ————. Ueber die Methoden zum Nachweis der Plasmaverbindungen. Ber. Deut. Bot. Ges. **15**: 166–177. 1897.
51. ————. Notiz über die Bedeutung der Plasmaverbindungen für die Propfbastarde. Ber. Deut. Bot. Ges. **32**: 447–453. 1914.
52. ————. Morphologische und physiologische Analyse der Zelle. 1920.
53. MÜHLDORF, A. Ueber die Plasmodesmen in den Endospermzellen der Umbelliferenfrüchte. Bul. Fac. Stiinte, Cernăuti **7**: 196–234. 1933.
54. ————. Das plasmatische Wesen der pflanzlichen Zellbrücken. Beih. Bot. Centralbl. **56**(A): 171–364. 1937.
55. PFEIFFER-WELLHEIM, F. Ueber ein Silberimprägnierungsverfahren zur Darstellung der Plasmodesmen in einigen Endospermengeweben und bei Moosblättchen. Zeits. Wiss. Mikr. **41**: 325–334. 1924.
56. PISKERNIK, A. Die Plasmaverbindungen bei Moosen. Oest. Bot. Zeits. **64**: 107–120. 1914.
57. POIRAULT, G. Recherches anatomiques sur les cryptogames visculaires. Ann. Sci. Nat. Bot. VII. **18**: 113–256. 1893.
58. PRIESTLEY, J. H. The physiology of cambial activity. II. The concept of "sliding growth." New Phytol. **29**: 96–140. 1931.
59. QUISUMBING, E. Continuity of protoplasm in endosperm cells of *Diospyros.* Bot. Gaz. **80**: 439–449. 1925.
*60. RUSSOW, E. Ueber die Perforation der Zellwand und den Zusammenhang der Protoplasmakörper benachbarter Zellen. Sitzgsb. Naturf. Ges. Univ. Dorpat **6**: 562. 1883.
*61. SCHAARSCHMIDT, J. Kommunikation von Protoplasten und das Vorkommen interzelluläres Protoplasma. Zeits. Wiss. Mikr. **1**: 301. 1884.
* Not seen, cited from Mühldorf.
62. SCHUHMACHER, W., AND HALBSGUTH, W. Ueber den Anschluss einiger höheren Parasiten an den Siebröhren der Wirtspflanzen. Ein Beitrag zur Plasmodesmenproblem. Jahrb. Wiss. Bot. **87**: 324–355. 1939.
63. SHARP, L. W. An introduction to cytology. 1926.
64. SINNOTT, E. W., AND BLOCH, R. Changes in intercellular relationships during the growth and differentiation of living plant tissues. Am. Jour. Bot. **26**: 625–633. 1939.
65. STOPES, M. C., AND FUJII, K. The nutritive relations of the surrounding tissues to the archegonia in Gymnosperms. Beih. Bot. Centralbl. **20**: 1–24. 1906.
66. STRASBURGER, E. Ueber Plasmaverbindungen pflanzlicher Zellen. Jahrb. Wiss. Bot. **36**: 493–601. 1901.
67. ————. Das botanische Praktikum. 7th Ed. 1923: 586–687.
68. STUDNICKA, F. R. Die Organisation der lebendigen Masse. W. Höllendorf: Handb. Mikr. Anat. Menschen, 1929.
69. SYKES, M. Anatomy and histology of *Macrocystis pyrifera* and *Laminaria saccharina.* Ann. Bot. **22**: 291–325. 1908.
70. TANGL, E. Ueber offene Communicationen zwischen den Zellen des Endosperms einiger Samen. Jahrb. Wiss. Bot. **12**: 170–190. 1879.
71. ————. Zur Lehre von der Continuität des Protoplasmas im Pflanzengewebe. Sitzgsb. Akad. Wiss. Wien **90** (Abt. 1): 10–38. 1884.
72. ————. Studien über das Endosperm einiger Gramineen. Sitzgsb. Akad. Wiss. Wien **92** (Abt. 1): 72–100. 1885.

73. Thoday (Sykes), M. G. On the histological relation between *Cuscuta* and its host. Ann. Bot. **25**: 655–682. 1911.
74. Tröndle, A. Eine neue Methode zur Darstellung der Plasmodesmen. Verh. Schweiz. Naturf. Ges. **44**: 213–217. 1913.
75. Tunmann, O. Pflanzenmikrochemie. 2nd Ed. 1931.
76. Tupper-Carey, R., and Priestley, J. H. The cell wall in the radicle of *Vicia Faba* and the shape of meristematic cells. New Phytol. **23**: 156–158. 1924.
77. Wulff, Th. Plasmodesmenstudien. Ark. Bot. **5**: 1–20. 1905.
78. ———. Plasmodesmenstudien. Oest. Bot. Zeits. **56**: 1–8, 60–69. 1906.
79. Zimmermann, A. Beiträge zur Morphologie und Physiologie der Pflanzenzelle. I. 1893.
80. ———, and Schneider, H. Die botanische Mikrotechnik. 1922.

CHROMOSOME MORPHOLOGY IN MAIZE AND ITS RELATIVES

A. E. LONGLEY
Bureau of Plant Industry

This review of the morphology of the chromosomes of maize and its relatives includes representatives from three genera of the tribe Tripsaceae. These genera, *Tripsacum, Euchlaena* and *Zea,* are endemic to the western hemisphere, and to many are better known by their common names, gamagrass, teosinte and maize, respectively.

The branch of cytology that treats with chromosome morphology is still in its infancy. Thanks, however, to the use of aceto-carmine stains on smear preparations of the pollen mother cells the chromosomes of maize are probably better known today than those of any other plant.

The morphological features of somatic chromosomes of maize and its relatives, which lend themselves to comparative studies under the microscope, are length, primary and secondary constrictions, the relative length of the arms, and the satellites. The meiotic chromosomes have additional features, such as chromomeres and knobs, while at this phase the primary constrictions are bodies with definite characteristics, and the satellite chromosome is made more prominent by the presence of a visible nucleolus-organizing body.

Enumeration and description of the various chromosome types found in maize and its relatives would seem as useless as classifying the various leaf shapes of sassafras if the chromosomes, both individually and collectively, were not related to the genetics and taxonomy of this plant group. In addition, they have given a clearer insight into the life history of these bodies that have come down from the dark ages of the past and may exist and be subject for studies in the years to come.

CHROMOSOME MORPHOLOGY

Two decades ago the relative lengths of the chromosomes of an appreciable number of maize strains were given (20). Similar measurements of somatic chromosomes, made ten years later (36), showed that the length range from the longest to the shortest chromosomes is from 4.5 to 2 microns, a difference too small to differen-

tiate with certainty between chromosomes of the normal diploid complement and the supernumerary chromosomes characteristic of some plants in several maize strains. However, such measurements did serve to show that the extra chromosomes are of the shortest type.

The paired chromosomes of the pollen mother cells at diakinesis were repeatedly used (19, 20, 21, 36) for comparing the relative size and shapes of maize chromosomes. Here again the investigators did not detect a constant difference between the smaller chromosomes of the normal set and the supernumerary chromosomes. Later it was shown (36) that there is no fixed number of O-, V-, or rod-shaped chromosomes, and the logical conclusion was that maize chromosomes at diakinesis are not favorable for identification on the basis of their form.

Darlington's theory that chromosomes are held together at diakinesis by chiasmata serves to explain the varying chromosome forms of maize at this phase, since neither the number nor position of chiasmata is constant for any chromosome and consequently the number of O-, V-, or rod-shapes will not be constant.

Chromosome morphology of maize really began with the discovery that in the first division of the microspore every chromosome of maize is morphologically identifiable (26).

The chromosomes in the microspore were never extensively used in morphological studies, because almost simultaneously the same author (27, 29) discovered a still more favorable phase early in the first meiotic division of the pollen mother cells. At this phase the chromosomes are from ten to twenty fold longer than the corresponding chromosomes in somatic cells, and many differentiating characteristics can be clearly seen in aceto-carmine preparations.

The mid-prophase chromosomes of the first reduction division have been subjected to a careful study for the past ten years. In maize, teosinte and *Tripsacum* the chromosomes at this phase seem to fill only a small part of the nucleus, and as they loosen from the synaptic skein they are frequently found so loosely intertwined that in spite of their length all bivalents may be traced chromomere by chromomere throughout their whole length. Equally important to morphologists is the fact that iron-aceto-carmine stains the mid-prophase of maize and its relatives so that even minute morphological features are visible.

Nine of the ten linkage groups were well known at the time of the discovery that each chromosome of maize could be identified. To weld the link between genetics and cytology it seemed important to assign to each chromosome a number that would serve to identify each chromosome and its corresponding linkage group. Consequently, the chromosomes were numbered from 1 to 10 (30), beginning with the longest as No. 1 and ending with the shortest as No. 10. These numbers have proven satisfactory labels for both the chromosomes and their linkage group and have been taken over to include their homologues in teosinte.

In the following review attention will be focused mainly on the mid-prophase chromosomes of the first reduction division of the pollen mother cells.

CHROMOSOME LENGTHS

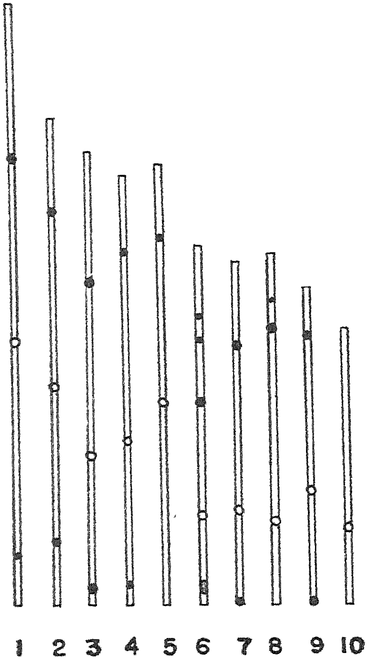
It is known that the length of a chromosome changes with each progressive step. Consequently, length measurements of mid-prophase chromosomes vary. The longest measurements are of chromosomes that have just emerged from the synaptic skein, and the shortest measurements useful for morphological comparisons are of chromosomes contracted to approximately half that length or contracted to the phase where the morphological characters begin to lose their sharpness.

There is much to suggest that the contracting process may not take place simultaneously in all chromosomes or even in different sections of the same chromosome. This possibility makes it necessary to obtain a large number of measurements of each chromosome so as to have dependable data to show the relative length of the ten chromosomes.

Averaging a large number of measurements of each chromosome of both maize and teosinte (22, 24, 25) gives a mean length for each that will serve for comparative purposes. Chromosome 1, in both maize and teosinte, is approximately 82 microns long, and chromosome 10 is approximately 37 microns long. These lengths, and the lengths given for the longest and shortest chromosomes in somatic tissue, stand in approximately the same relation to each other.

The relative lengths of the monoploid chromosome set of maize, teosinte and *Tripsacum* are shown diagrammatically in figures 1, 2 and 3, respectively. The length of each chromosome of maize and

teosinte is from sufficient numbers to reduce the error to a reasonable figure, but the length of each of the 18 chromosomes of *Tripsacum* is from so few measurements that the positions assigned in figure 3 are subject to revisions.



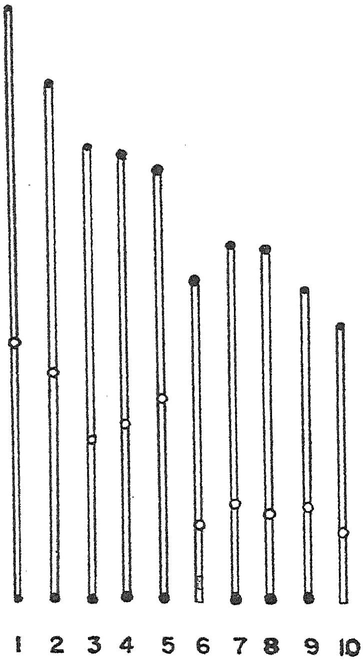


FIG. 1 (*left*). Diagram of the 10 chromosomes of maize showing relative mean length at mid-prophase I, all known knob positions (black), nucleolus-organizing body (shaded) and centromeres (circles). × 1000.

FIG. 2 (*right*). Diagram of the 10 chromosomes of teosinte from southern Guatemala showing relative mean lengths at mid-prophase I, all known knob positions (black), nucleolus-organizing body (shaded) and centromeres (circles). × 1000.

A comparison of the lengths of the homologous chromosomes of maize and teosinte has led to the conclusion that they show no significant differences (25). The slight difference in length between chromosomes 6 of maize and of teosinte disappears when the two chromosomes are in the same cells of an F_1 hybrid (22). Other maize and teosinte homologues that gave no indications of a difference in length have, when they are side by side in the cells of an F_1 hybrid, occasionally shown slight differences. Even the much

shorter chromosomes of *Tripsacum*, when contained in the same cells of an F_1 hybrid with maize (34), were not recorded as differing in length from their maize homologues.

Length measurements of chromosomes are exceedingly useful, for both identification and comparative purposes, but they must be used with caution because of the uncertainty that chromosomes are in the same phase in sister cells. Also, there is the possibility of different chromosomes or parts of chromosomes in the same cell failing to

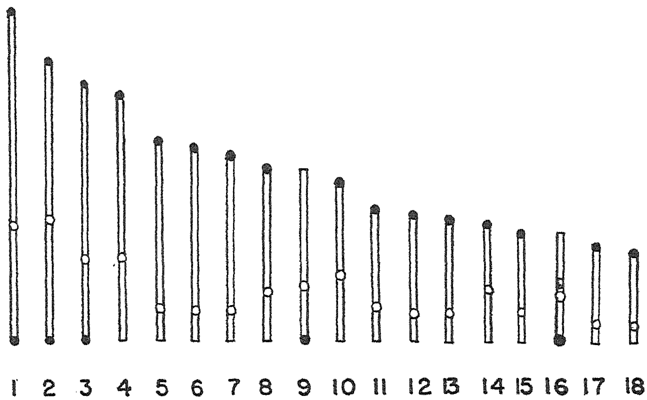


FIG. 3. Diagram of the 18 chromosomes of *Tripsacum floridanum* showing relative mean lengths at mid-prophase I, all known knob positions (black), nucleolus-organizing body (shaded) and centromeres (circles). ×1000.

shorten simultaneously. It may be that this non-simultaneous contraction has caused chromosome 6 of teosinte to always appear shorter than chromosomes 7 and 8 (25) when in a teosinte cell, but it is as long as its maize homologue (22) when in the cells of an F_1 maize teosinte hybrid.

A consistent premature shortening of B-type chromosomes is suggested to explain the fact that in somatic cells B-type chromosomes are approximately the same length as the shortest chromosomes of the normal set, while in mid-prophases they are only approximately 15 microns long as compared with 37 microns for the shortest chromosomes of the normal set.

B-type chromosomes are those chromosomes that were so puzzling in the earlier studies of chromosome numbers of maize. It was the discovery that they are distinct from the chromosomes of the normal monoploid set (37) and that they could be duplicated many times in

a cell without deleterious effect, which has served to explain why some maize plants have different chromosome numbers and are still normal in appearance.

Finally, there is the consideration of an abnormal form of chromosome 10. It has appeared in several strains of Indian corn from Guatemala, Mexico, Arizona and New Mexico. It is considered abnormal since it has only recently been discovered (22, 23), but its presence in Indian strains from these central regions of maize culture and its absence from improved maize strains may throw some doubt on its abnormal status.

The newly discovered form of chromosome 10 has an additional piece on the end of the long arm, approximately 12 microns in length. This additional piece is heavily laden with pycnotic material and gives this chromosome in its newer form a total length greater than that of chromosome 6.

THE CENTROMERE

The most conspicuous and constant body seen in the mid-prophase chromosomes of maize, teosinte and *Tripsacum* is the centromere or spindle fibre attachment region.

The centromere in aceto-carmine preparations is round or oval in outline, transparent or lightly stained, and swollen slightly beyond the margin of the adjacent threads. The centromere is useful for chromosome identification because it has a constant size, shape and position for each chromosome (27). Size and shape differences account in part for the fact that centromeres of some chromosomes are more prominent than those of others.

The position of the centromere on the chromosome may be median, sub-median, terminal or sub-terminal. In maize and teosinte the position of each centromere has been rather definitely fixed by extensive measurements. The position of each centromere is conveniently expressed by a fraction which shows the relative length of the short arm to the long arm or to the whole chromosome (22, 24, 25). This fraction, in both maize and teosinte of southern Guatemala, has the highest value when the arms are nearly equal in length, as in chromosome 5, and the lowest value when the arms are most unequal, as in chromosome 8 (25).

The position of the centromere is affected, to some extent, by the possibility that different sectors of the same chromosome do not

contract simultaneously, but its position is not affected by differences in length, due to phase differences or to non-simultaneous contraction of different chromosomes in the same cell.

The importance of a centromere in the life history of a chromosome has been well illustrated in the study of chromosome fragments. Only a chromosome fragment that includes a centromere is carried on from one cell generation to the next, and fragments without a centromere soon disappear (29, 33). A centromere, therefore, is essential to the life of a chromosome. On the other hand, the presence of two centromeres in what is equivalent to one chromosome (33) causes conflicts when this chromosome divides so that not one unit but two are produced by its division.

X-ray treatments have produced many changes in the normal chromosome or chromosomes. Many of these changes are not perpetuated, but one instance has served to show that a fragment of a centromere may function as a whole centromere (32, 41). This newly formed centromere and the associated chromatin eventually brought about the formation of a secondary trisomic plant, *i.e.*, a plant with a monoploid set of eleven chromosomes (42).

A peculiar feature of centromeres is the occasional fusion of two or more, and at mid-prophase exceptional figures may show as many as a half dozen centromeres fused into what appears to be one homogeneous body. This fusion, however, is only transitory, and it has never been demonstrated that the individuality of a centromere has been altered by such close associations with sister centromeres.

The conclusion, from extensive comparisons of the positions of centromeres of maize and teosinte chromosomes, is that there are no consistent differences in centromere positions for the homologous chromosomes of these two related groups (25).

CHROMOSOME ARMS

When the length of the whole chromosome and the position of the centromere have been discussed, any further treatment of arm length becomes superfluous. An intensive study of the features of the chromosomes has led, however, to a consideration of each arm and not the whole chromosome as a unit. The chromatic thread of a chromosome does not pass through the centromere; thus the monoploid set is made up of twenty and not ten chromatic threads. The consideration of each chromosome arm as an independent unit

has assisted materially in explaining the various positions and the various frequencies of the knobs on the chromosomes of maize and teosinte (24, 25).

CHROMOSOME KNOBS

Knobs are pycnotic swellings on the chromosomes of maize, teosinte and *Tripsacum,* most prominent at mid-prophase. They range in size from that of a prominent chromomere to swellings slightly over 2.5 microns across their longest axis. It may not always be possible to distinguish between a large chromomere and a small knob. In staining reaction with aceto-carmine, no difference can be detected between the pycnotic material of a chromomere and that contained in the knobs. The general rule has been to classify a prominent chromomere as a knob only when its position on the chromosome coincides with the position known to bear a prominent knob in other strains.

This introduces the one feature of knobs that has made them so useful in chromosome identification, namely, that knobs when present occupy a constant position on a chromosome (29). For example, chromosome 1 of maize may be without knobs, but if knobs are present there will be one on the long arm at a point approximately one-fourth the length of the chromosome from the end and one on the short arm at a point approximately one-sixth the length of the arm from the end and at no other positions.

The positions of the knobs on the chromosomes for maize, teosinte and *Tripsacum* are shown diagrammatically in figures 1, 2 and 3.

A survey of the number, size and positions of knobs of many strains of maize and teosinte has shown that, although the position of each is constant, both the number and size of knobs may vary. Some knobs are almost universally present on the chromosomes of all strains, while others are present only in a few strains (24, 25). Thus some knobs have a much higher frequency of occurrence than others.

Comparing the size of different knobs is a complicated problem, since it involves volume calculations of different shaped bodies. Some knobs seem to be characteristically large and prominent, but it is not unusual to find in other strains that such knobs are much smaller, and so the size of a knob at any particular position is not constant.

In maize, knobs have been found at 18 different positions on the chromosomes. It has been suggested (24) that these 18 knob-forming points exist on the 10 chromosomes of all maize plants. When they are filled with pycnotic material, they are visible, but in many strains they do not show, because no knob-forming pycnotic material is available.

An explanation of the differences in frequency of the different knobs of maize and teosinte was suggested when the 20 chromosome arms were arranged in the order of their length, and all the knob positions were marked on them. These diagrams (24, 25) showed that there are no knobs close to the centromere, and the knobs nearest to or farthest from the centromere, relatively speaking, are the knobs that occur least frequently. These facts lead to the conclusion that on all arms of an appreciable length there is a point most favorable for knob formation, and the knob-building power of a knob-forming point decreases as its distance from the most favorable point increases. This difference in knob-building power of points at different distances from the centromere was interpreted to mean that each chromosome arm possesses a gradient.

The chromosomes of teosinte from southern Guatemala were found to have their shortest and longest arms without knobs. All medium length arms are terminated by knobs. Comparing the position of knobs having approximately the same frequency and occurring on chromosome arms of teosinte and maize that are approximately equal in length, it was found that knobs are nearer the centromere when on the arms of maize than when on the arms of teosinte chromosomes. This observation led to the conclusion that the gradient controlling knob formation for this group of teosinte is different from that for maize (25).

All varieties of teosinte from northern Guatemala have terminal knobs, but the number of knobs is greater than on plants from farther south. A comparison of the knobs of the two groups of teosinte showed that some of the short arms that are knobless in the southern group are terminated by large, frequently occurring knobs when in the northern group. This comparison failed to show any significant differences in the position or number of knobs on the 12 arms of the 6 longest chromosomes of these two teosinte groups (25).

Continuing this comparison, it was found that the position and

frequency of the knobs on the 8 arms of the 4 shortest chromosomes of teosinte from northern Guatemala have the knob frequencies which might be expected if these same knob-forming positions were present on the 4 shortest chromosomes of maize. This observation suggested that the 4 shortest chromosomes of teosinte from northern Guatemala were controlled by a gradient similar to that of maize, while the 6 longest chromosomes were controlled by the gradient similar to that of the teosinte of southern Guatemala (25).

If the view that a gradient along each chromosome arm causes the differences in the positions of knobs in maize and teosinte, it would seem that the gradient controlling the 20 chromosome arms of maize is different from that controlling the 20 arms of teosinte from southern Guatemala. Teosinte from northern Guatemala with 8 arms controlled by a gradient similar to that of maize and 12 arms with a gradient similar to that of teosinte from southern Guatemala occupies an intermediate position (25).

A change in gradient of one chromosome or a group of chromosomes, it is thought, might be easily brought about by one or a few mutations that would not visibly alter a chromosome beyond, causing a new set of knob-forming points to become active. Thus in teosinte of northern Guatemala, many more knobs are formed because several knob-forming points on the 4 shortest chromosomes that were inactive in teosinte from southern Guatemala have by a change in gradient become active (25).

There is a relationship between knob size and knob frequency in Guatemalan teosinte (25). The least frequently occurring knobs are smaller than knobs occurring with a high frequency. Such relationship between knob size and knob frequency in maize is less definite. It is true that the infrequently occurring knobs near the end of the short arms of chromosomes 3 and 4 are large, in some strains heavily impregnated with pycnotic material,[1] but miniature in others that seem to be carrying less pycnotic material. On the other hand, certain knobs, such as the one on the long arm of chromosome 5 and the two better known knobs on chromosome 6, seem to be more limited in the amount of pycnotic material they can absorb than other knobs which occur far less frequently. Thus, it would seem that some internal knobs carry with them a restriction which limits the

[1] "Pycnotic material" is used in its broadest sense so as to include all material of the chromosome that stains with aceto-carmine.

quantity of pycnotic material they can absorb, while the terminal knobs of teosinte have a more uniform size, and knobs are small or large depending upon their position and the amount of pycnotic material present.

In teosinte it has been shown that knob-forming pycnotic material is most abundant in strains from southern Guatemala, slightly less in strains from northern Guatemala and almost completely absent from the perennial species of Mexico (22, 25).

The situation is similar and more definitely established for maize, the center for strains with abundant knob-forming pycnotic material is less restricted and includes Guatemala, Mexico, Arizona and New Mexico, and knobs become less abundant in strains as one goes north or south of this central region (24, 34).

The amount of knob-forming pycnotic material in a plant varies from enough to fill many knob-forming points to almost zero in both maize and teosinte. The presence or absence of this material, however, would, on superficial examination, seem insignificant, since teosinte is teosinte and maize is maize, whether knobs are present or absent.

A recent unpublished experiment (Longley) has shown that chromosomes in megaspore cells heavily laden with knob-forming pycnotic material have some advantages over cells containing less material. Does this signify that the abundant pycnotic material is associated with a high survival value? If so, the ability to survive must be strongest in plants with many knobs. Consequently, *Tripsacum* and teosinte from southern Guatemala and maize from Guatemala, Mexico, Arizona and New Mexico have a high survival factor or factors.

Maize plants have been produced that combine the large quantity of knob-forming pycnotic material of one strain and the almost complete absence of knob-forming pycnotic material of another strain. The effect of an intermediate quantity of pycnotic material in these F_1 plants on knob formation is that some of the knob-forming points in unfavorable positions fail to absorb pycnotic material.

The experiment also should have served as a test of those knob-forming positions favorably located for absorbing knob material but prevented from doing so in the parent plants by the absence of pycnotic material. There are no published data to show that such knob

positions become fully active immediately, although some experiments have shown a partial restoration of activity. The conclusion is that, although all 18 knob-forming points are present on the chromosomes of all maize plants, these points may have become atrophied through disuse, and the restoration of this activity is slow.

The chromosomes of annual teosintes from Mexico show many knobs. The knobs are at positions identical with those of maize. These teosintes have terminal knobs on only those arms that are terminated by knobs in both maize and teosinte from Guatemala. Most authorities look upon these internal knobs of Mexican teosinte as coming from maize through hybridization. This explanation is borne out by so many facts that it is seldom questioned. It does not seem impossible, however, to derive teosinte similar to some now growing in Mexico directly from Guatemalan strains by mutation, since one or a few mutations might suffice to change the chromosome gradient so that knobs no longer are formed at the terminal points but at other more favorably located internal knob-forming points.

One characteristic of knobs is their frequent tendency to stick together (30). In teosinte with terminal knobs, practically all knobs may be united into one large or several smaller bodies (22). Environment may play an important rôle in bringing about this agglutination, but this physiological problem has never been investigated under controlled conditions. Observations, however, have suggested that when knobs are equal in size the tendency to fuse decreases as the distance of the knobs from the end of the chromosome increases, and that for knobs in the same position the larger tend to fuse more frequently than the smaller. Might not such a behavior, if substantiated, indicate that there is a protecting sheath that becomes thinner as the distance from the centromere increases and also becomes thinner, due to the bulging caused by the knob within?

In conclusion, it may be said that knobs, when present, are useful morphological characteristics for the quick identification of chromosomes. They have aided in identifying the different chromosomes of the monoploid set of maize and teosinte, in distinguishing the chromosomes of maize from those of teosinte from Guatemala, and in distinguishing teosinte from different localities, such as Mexico, northern Guatemala and southern Guatemala.

THE NUCLEOLUS-ORGANIZING BODY

The nucleolus-organizing body is a characteristic morphological feature of chromosome 6 of maize and teosinte and of the third shortest chromosome of *Tripsacum*. The tetraploid perennial teosinte has two such bodies at mid-prophase, since chromosome 6 is duplicated and in this teosinte these bodies may either unite in the formation of a single nucleolus or remain apart to form two.

This nucleolus-organizing body has been well described (31) and pictured. It is made up of two distinct parts, the thin practically colorless thread or ribbon that runs across the surface of the nucleolus and the adjacent deeply staining body that is indistinguishable from a knob. The colorless part may in somatic cells extend across so much of the surface of the nucleolus that the connection between the short chromatic thread and the main part of the chromosome is obscure. Such a separation of a small fragment from the main part of the chromosome has given it the name satellite chromosome. At mid-prophase, however, the almost colorless part of the nucleolus-organizing body is much shortened, and the short chromatic thread beyond appears as a short piece of the chromosome similar in character to other sections, thus making the term satellite seem less appropriate, although it may always be retained as a label for chromosome 6.

The term satellite chromosome, that has become almost universally accepted as designating the chromosome attached to the nucleolus, becomes clearly inapplicable when used to describe the third shortest chromosome of *Tripsacum* or the longest chromosome of sorghum (22), both of which have their nucleolus-organizing body at the mid-point of the chromosome and not near the end, as in maize and teosinte.

The pycnotic part of the nucleolus-organizing body is observed only at those division phases which show knobs at other points on the chromosomes. It is also well known that in sorghum and other plants the nucleolus-organizing body is not characterized by the pycnotic area; consequently, the lightly staining area may be the true organizing part, and the pycnotic concentration would seem to be of secondary importance.

OTHER MORPHOLOGICAL FEATURES

There are other pycnotic concentrations similar, though less

prominent, than the one that is part of the nucleolus-organizing body, which cannot be classed as knobs but which have served to identify certain maize and teosinte chromosomes.

Two of the most constant and conspicuous of these darkly staining areas are on the long arm of chromosome 7 and the short arm of chromosome 10, adjacent to the centromere. The short arm of chromosome 3 is distinctly stouter, due to heavy concentrations of stainable material, than the short arm of the other long chromosomes. The region around the centromere in all chromosomes is usually more pycnotic than the more distant regions, but the above three examples are so constant and characteristic that they frequently serve as aids in the identification of these 3 chromosomes.

The chromomeres of the mid-prophase chromosomes of maize and its relatives have never been shown to be constant in size and number for the whole length of a chromosome. Chromomeres have been interpreted by students of the chromosomes of other plants as kinks in a spirally twisted chromosome, an interpretation that has never been confirmed or refuted, even after critical and prolonged studies of maize chromosomes. In maize the chromomeres appear as nothing more or less than small concentrations of pycnotic material.

Chromomeres resemble somewhat a string of beads occurring about a micron apart over the whole length of the chromosome. The small sector of chromosome 6 beyond the nucleolus-organizing body in maize and teosinte is 4 chromomeres long, and even the loss of one chromomere has been detected (12). No one, however, has attempted a complete mapping of the chromomeres of a single chromosome, possibly because their constancy in size and number has not been demonstrated. It is not exceptional to find adjacent chromomeres unequal in size and to find sectors in which the chromomeres of the two parallel chromosomes are not in pairs but are alternate in position.

These exceptions to the expected complete homology of the two chromosomes may be overemphasized, since, if differential shortening or tension is acting upon the two adjacent threads, the homologous chromomeres might easily be drawn out of place.

A few prominent chromomeres have been observed so constantly at certain points of some chromosomes that they have served to identify these chromosomes in certain strains of maize. Two examples are the large chromomere terminating the short arm of chro-

mosome 1 and the large chromomere on the long arm of chromosome 6 at a point approximately one-third the length of the arm from the centromere.

The association of homologous chromosomes at mid-prophase is affected by certain changes in the chromosomes, such as deletions, inversions and translocations. For instance, a certain type of semi-sterility in maize suggested that a change had taken place in two pairs of chromosomes (2). Later studies of diakinesis in semi-sterile plants showed rings of 4 chromosomes, and studies of the mid-prophase chromosomes showed which two chromosomes had reciprocal translocations and were associated with their normal homologues in a ring or chain of 4 (1–15, 18, 27–30, 38, 39).

A chromosome with a deletion, even if the lost piece is small, will be clearly visible when paired with its normal homologue in most mid-prophase figures. The chromosome with a terminal deletion is most readily detected, since the two unequal length chromosomes will have an unpaired piece at one end (17, 29, 30, 40, 41, 44, 45). The length, or even the presence of a terminal deletion, may be undetectable in some instances if the single thread folds back on itself and pairs non-homologously.

Internal deletions in a chromosome are detected when paired with a normal chromosome by the presence of single thread loops which shorten the normal chromosome, thus bringing together the homologous parts of the normal and the modified chromosome (29, 30, 33). A similar adjustment in length is sometimes seen when a chromosome with a knob is paired with a knobless homologue, the knob in such instances frequently standing out to one side in much the same manner as a single thread loop (22).

Inversions, when tested against the normal chromosomes, cause the formation of double loops, except when they are short and non-homologous pairing hides the affected sectors (29, 30, 33). If crossing-over occurs in a double loop, two centromeres are together in one continuous thread and the complementary pieces are left without a centromere. The presence of chromosome bridges and fragments at anaphase is the outcome of inversions and cross-overs. Bridge configurations have served to demonstrate that crossing-over occurs at the four strand stage (33) of the first division prophase.

F_1 hybrids of maize and teosinte from southern Guatemala frequently show as many as three or four bridges at the first division

anaphase, indicating that several of the chromosomes of maize differ in the linear order of the chromatin of several sections from that of some teosinte strains.

Reciprocal translocations disturb, even more than inversions, the linear order of the genes. In the effort to bring about pairing of homologous sectors, the pair of chromosomes with translocations when paired with their normal homologues gives a ring of four chromosomes. Such figures have not proved very helpful to determine the length of the translocated sectors, since it is impossible to tell where homologous pairing begins and non-homologous pairing ends (13, 30). Strains of maize have been isolated that are homozygous for reciprocal translocations, but it has never been demonstrated that such strains will persist without special care.

Chromosome fragments are pieces of the normal chromosomes that have become detached. All such fragments are difficult to retain. Those that do not contain a centromere are lost almost immediately, those with a centromere frequently have been carried on for several generations. Their effect on the genetic ratios of the characters involved and on the gross morphology of the plants has been extensively investigated (32, 42). Not only is the life of a fragment containing a centromere short, but it has been found to both decrease and increase in size through deletions and reduplications of shorter or longer sectors. These changes were found to occur in somatic, as well as meiotic, divisions (32, 42).

The absence of pairing at mid-prophase in most instances is thought to be due to absence of homology. When maize and *Tripsacum* chromosomes are in the same cell, very little pairing takes place between the chromosomes from these related genera. The pairing of small sections of maize with *Tripsacum* chromosomes does signify that there are some homologous pieces, barring the possibility of non-homologous pairing entering into these associations.

Thus chromosome associations, in combination with identifying morphological features, have aided in the study of the relationship of chromosomes and parts of chromosomes within a genus and between related genera.

A study of the morphological features of the chromosomes of maize and its relatives may become as fascinating as a mathematical or physical problem. The problem becomes more involved, however, when associated with the allied studies of genetics, taxonomy and life history of the chromosomes.

CYTOGENETICS

Rhoades and McClintock (42) have reviewed the cytogenetics of maize up to 1935. From their summary are taken some of the results of cytogenetic studies:

1. The placement of many of the known genes into 10 linkage groups corresponding to the 10 morphologically identifiable chromosomes.
2. The placement of specific genes at definite points within the physical chromosome.
3. Cytological and genetical proof of chromatial crossing-over.
4. The reduction in normal crossing-over resulting from non-homologous association.
5. The relationship between semi-sterility and reciprocal translocations and the importance of different sectors of the chromosome to viability of the gametes by means of haplo-viable and haplo-inviable deficiencies.
6. The meiotic behavior of ring-shaped chromosomes resulting in genetic variations.
7. The correlation of pycnotic chromatin with genetic inertness.

The more recent cytogenetic studies of maize have continued to utilize deficiencies, translocations and inversions to consolidate the foregoing conclusions and have resulted in the more definite locations of several genes.

Specific translocations of maize chromosomes have been used to test the homology between maize and teosinte chromosomes (4). This study gave evidence that chromosomes 5 of maize and of Florida teosinte have slight structural differences and that the second pair showing slight differences is possibly either chromosome 8, 9 or 10. The general conclusions, however, were that gene changes may have been chiefly responsible for the differentiation of maize and annual teosinte.

MORPHOLOGY AND THE LIFE HISTORY OF THE CHROMOSOME

The one essential feature of the chromosome, which is necessary to its division and life, is the centromere. Usually there is considerable chromatic material associated with the centromere, but certain maize chromosomes have been found that are little more than a centromere (23, 37). The one requirement of the whole monoploid chromosome set is that it contain the chromatin essential for

normal development. Either a deficiency or a duplication (43) of the essential chromatin becomes a handicap to the pollen when in competition with normal pollen. A similar effect is noticed in a plant that has lost or gained a chromosome or part of a chromosome belonging to the normal complement. This rule would seem to have some exceptions when it includes polyploid forms, although many autotetraploids have been found to be inferior to the diploid parent in fertility and ability to survive.

X-ray treatments are widely used in producing changes in the amount and arrangement of the chromatin, such as deletions, inversions and translocations.

No maize strain carrying an appreciable deletion of chromatin from the normal diploid complement has been maintained for any length of time. Inversions, on the other hand, do not deprive the plant of any essential chromatin, and unless cross-overs lead to later deletions an inversion or inversions may not be detrimental to a plant. Consequently, it should not be difficult to maintain inversions in the homozygous condition. Reciprocal translocations are more drastic changes in the linear order of the chromatic material, but even these have been obtained and maintained in their homozygous condition. Some recent studies of maize strains from different geographical regions indicate little, if any, difference in the chromosome structure in maize from widely separated regions (16). This fact suggests that the normal arrangement of the chromatin material has the greatest survival value.

A comparison of the chromosomes of maize and teosinte from northern Guatemala shows that in F_1 hybrids there are several bridges at the first division anaphase. Thus there is suggested that at least two different arrangements of the essential chromatin have a satisfactory survival value.

That there is a chromosome gradient which controls knob formation has served to explain the shift in knobs from terminal points to points within the chromosome or points not so far removed from the centromere. It has been suggested that one or a few mutations would bring about a change in gradient sufficient to change the four shortest chromosomes of teosinte of northern Guatemala from the type found in teosinte of southern Guatemala or to change all the chromosomes of teosinte from southern Guatemala to the type found in maize. Such a change could be brought about without

altering the morphological features of the chromosomes, other than the position of the knobs. This suggestion would be strengthened if it could be shown that other factors related to the chromosomes were changed with a change of gradient. A change in linear order of the chromatin has been produced many times artificially and unquestionably has occurred in nature. That such changes have not persisted (16) would suggest that a change in position of a sector of a chromosome is associated with change in gradient for that section, and in its new position its effect on the survival of the plant is altered, or, in other words, the effectiveness of a gene is related to its position on the chromosome. Thus, the same chromosomes under different gradients may give distinctly different plants.

CHROMOSOME MORPHOLOGY AND TAXONOMIC RELATIONSHIP

In contrasting the morphological characteristics of *Tripsacum,* teosinte and maize chromosomes, it is difficult to give the proper weight to their similarities and differences.

Tripsacum, with a basic chromosome number of 18, is definitely set apart from both teosinte and maize with their basic numbers of 10. All three genera are thought to have come from a remote common ancestor, but no one has attempted to guess what its basic chromosome number must have been.

The chromosomes of maize fail to pair, or at best pair poorly, with the chromosomes of *Tripsacum,* but with teosinte the pairing is normal for some strains (35), although somewhat irregular for others. When the monoploid sets from all three genera are together in one cell, the maize and teosinte chromosomes pair to form bivalents, with only an occasional trivalent, due, it was thought, to a *Tripsacum* chromosome loosely paired with a maize chromosome (34). Thus chromosome association substantiates the close relationship between maize and teosinte, and the distant relationship between maize or teosinte and *Tripsacum* that is suggested by their basic chromosome numbers.

A comparison of the length and centromere positions of homologous chromosomes suggests no striking differences between maize and teosinte. The chromosomes of *Tripsacum,* however, are not well enough known to make a fair comparison with either of these related genera.

One characteristic morphological feature that is usually asso-

ciated with maize, teosinte and *Tripsacum* is the pycnotic material that forms knobs on the chromosomes. These knobs are terminal on the chromosomes of *Tripsacum* and of teosinte from Guatemala, as contrasted with the frequent internal knobs on the chromosomes of maize and teosinte from Mexico. Knob position, therefore, is a differentiating feature and one that would seem to place teosinte in an intermediate position between *Tripsacum* and maize.

If the positions of the knobs of some strains of teosinte are similar to those of *Tripsacum* and in other strains are similar to those of maize, to what can the difference be attributed?

A critical comparison of some strains of teosinte from Guatemala shows that in the northern group knobs are found nearer the centromere than in the southern group. This is thought to be due to an invisible change in the four shortest chromosomes, a change in gradient which affects these eight chromosome arms so that knobs are no longer held off so far from the centromere. The twelve arms of the six longest chromosomes, however, have still retained the knob-forming characteristics belonging to the teosinte from the South.

This change in gradient in four of the northern teosinte chromosomes suggests a step towards maize, since these chromosomes appear more like maize chromosomes than like their homologues in the teosinte from southern Guatemala. Maize, however, has gone one step farther, and all ten chromosomes have gradients that allow knob formation at points much nearer the centromere than the knobs on the chromosomes of southern Guatemala teosinte.

Chromosomes of the several types of annual teosinte found in Mexico have knobs at positions identical with those found on maize chromosomes. It is difficult to say to what extent these internal knobs have come through recent hybridization with maize and to what extent they have come through the mutation path from teosinte ancestors with terminal knobs. It does not seem unreasonable that gradient changes in the chromosomes, brought about by one or a few mutations, might produce teosinte very similar to that existing in Mexico today.

Thus it seems reasonable to interpret knob position data to mean that *Tripsacum* and teosinte with terminal knobs are the most primitive representatives of this group. Through mutations teosinte chromosomes have taken on new characteristics, and new forms of teosinte have arisen, one or some of which might have been the ancestor of maize.

A characteristic of the pycnotic material that forms knobs is that it is present in different amounts in different strains of teosinte and maize, a characteristic that may be completely independent of the positions at which knobs are formed. Thus two plants with the same chromosome gradient may have few or many knobs, depending upon the amount of pycnotic material available.

In maize the largest amount of pycnotic material is found in Indian strains from Guatemala, Mexico, Arizona and New Mexico. This amount decreases to the vanishing point as the distance north and south of this central region increases (23, 34).

In teosinte the largest amount of knob-forming pycnotic material is found in strains from southern Guatemala. It is slightly less in strains from northern Guatemala and is practically absent from perennial teosinte of Mexico.

It has been thought that the knob-forming pycnotic material in maize and teosinte came from *Tripsacum* (34) and consequently that chromosome knobs of maize signify a strong *Tripsacum* admixture. There is, on the other hand, the possibility that a large amount of knob material signifies a close relationship to a primitive type and that the primitive types are *Tripsacum,* teosinte from southern Guatemala, and maize from the Central American region.

Decrease in the amount of knob material suggests a removal from the primitive type, but, since all three genera have representatives with a large amount of knob material, this decrease would seem to have followed the separation that produced these three genera.

Knob position seems to be the only morphological feature on the chromosomes of these three genera that has afforded sufficient data to suggest the nature of the relationship between *Tripsacum,* teosinte and maize. *Tripsacum* is at the base of the tree with its terminal knob, maize is in the branches with its internal knobs, and teosinte is the connecting link.

Therefore, the knobs on the chromosomes of teosinte are the only clue that morphological studies have contributed towards solving the relative positions of *Tripsacum,* teosinte and maize. The variation in knob position in teosinte is thought to be due to changes in gradient along the chromosome arm that might have been produced by a few simple mutations. These changes have not affected the linear arrangement of the chromatin but have changed the effective-

ness of certain points so that knobs are formed nearer the centromere and thus internal knobs are not excluded. Thus, the least modified teosinte is linked by its terminal knobs to *Tripsacum*, and the more modified teosinte has chromosomes indistinguishable from maize.

One must not overlook the fact that there have been changes in the linear arrangement of the chromatin, such as inversions, while the teosinte chromosomes were becoming more maize-like, but these inversions are too few and too limited in extent to account fully for the changes of many knobs from terminal to internal positions.

Just when maize and teosinte diverged is difficult to say. The fact that the chromosomes of teosinte from northern Guatemala are more nearly homologous with maize than those from southern Guatemala suggests that maize branched off from teosinte after, rather than before, mutations entered to change the chromosome gradient.

Thus, it is thought that the morphological data may be made to harmonize with a proposed family tree for the Tripsaceae in the following manner:

The abundant pycnotic knob-forming material and the terminal knobs on the chromosomes of *Tripsacum* suggest that this wild plant stands near the base of the tree. The same characteristics are found in teosinte from southern Guatemala and suggest that its position is on the trunk of the tree. A decrease in the pycnotic material and the presence of several chromosomes with knobs much nearer the centromere than found in teosinte of southern Guatemala suggest that teosinte from northern Guatemala is on a major branch of the tree. That other annual teosintes, with their knobs near the centromere, may exist is suggested. Some of these teosintes may be still growing in Mexico today, although unquestionably they have been contaminated by the maize that is growing in regions near them. Such teosintes, and even teosintes without knobs on their chromosomes (perennial teosintes), constitute the finer branches of the tree. It has been suggested that changes in chromosome gradient have brought about these diversifications in teosinte and that from one of these branches primitive maize with abundant knobs developed.

It is recognized that chromosome morphology has not contributed indisputable evidence to the taxonomic relationship of these three genera of the Tripsaceae. Terminal knobs have been found to be

typical of chromosomes of all the least modified wild species. The transition from terminal knobs far from the centromere to knobs close to the centromere and finally to internal knobs would seem to be associated with a transition from purely wild strains to strains less adapted to the wild and finally to strains requiring man's protection for their existence. This transition does not necessitate a change in the linear arrangement of the chromatin, since it has been suggested that a change in gradient brings about a change in the effectiveness of the chromatin at the different points along each chromosome arm.

That maize might be derived directly from teosinte seems possible, but the morphological data do not exclude the possibility that some unknown grass has entered into its ancestry. The maize of today has some extraneous chromatin, such as B-type chromosomes, and the additional piece found occasionally on chromosome 10. This chromatin has no counterpart in the purer teosintes. The search may have to go farther afield to account for these fragments of chromatin unknown to teosinte.

SUMMARY

Chromosome morphology is too new a field of investigation to expect that all the characteristic features of the chromosomes of maize and its relatives are now available. This newness is apparent in the evaluation of the data at hand. Some conclusions drawn from the data are well established. Some require additional confirmation before they can be generally accepted, while some data have given only encouraging leads and suggestions that will require additional research to bring about their confirmation or rejection.

The Morphological Features of Mid-Prophase Chromosomes

1. A mean chromosome length and a mean position for the centromere for each chromosome of maize and teosinte have been established.

2. Each chromosome is made up of 2 chromatic threads tied together by the centromere.

3. All chromosome threads have pycnotic enlargements known as chromomeres, about a micron apart over their whole length, some of which are sufficiently distinctive to be useful in chromosome identification.

4. A centromere may have a characteristic size and shape and

each divides its chromosomes into two arms, the lengths of which bear a constant ratio to each other.

5. One chromosome in each monoploid set of a diploid species has a nucleolus-organizing body.

6. Pycnotic swellings, known as knobs, occur at definite points on the chromatic threads of certain chromosomes of *Tripsacum* and certain strains of maize and teosinte.

7. Every chromosome of maize and teosinte is morphologically identifiable.

8. The close association of homologous parts of maternal and paternal chromosomes to form bivalents is the normal condition.

9. Chromosome deletions, inversions and reciprocal translocations cause visible changes in the normal bivalent association.

Effects Relating to Certain Morphological Features of Chromosomes

1. A loss or increase in the essential chromosome material is deleterious.

2. A centromere is essential for the retention of any chromosome or chromosome fragment.

3. Neither differences in length nor differences in the position of the centromere of homologous chromosomes of maize and teosinte are sufficient to differentiate between the chromosomes of these two genera.

4. The presence of chromatin bridges at anaphase of F_1 maize × southern Guatemalan teosinte shows a few differences in the linear arrangement of the chromatin of maize and of this teosinte subgroup.

5. The normal appearance of the bivalents in F_1 maize × northern Guatemalan teosinte and F_1 maize × annual Mexican teosinte shows full homology of the chromosomes of maize and the chromosomes of these teosinte subgroups.

6. The limited association of *Tripsacum* chromosomes with both maize and teosinte in F_1 hybrids indicates a difference as wide as the difference in their basic chromosome numbers.

7. The terminal chromosome knobs of *Tripsacum* and the internal knobs of maize differentiate the chromosomes of these two genera. The chromosomes of teosinte from Guatemala, when classified on knob positions, are more like those of *Tripsacum* than those of maize. However, the four shortest chromosomes of teosinte from

northern Guatemala are maize-like, although characterized by terminal knobs, while the chromosomes of annual teosintes of Mexico have knobs at the same position as the chromosomes of maize. Consequently, teosinte is in an intermediate position between *Tripsacum* and maize.

Suggestions Resulting from Morphological Studies

1. Each chromosome possesses a definite number of knob-forming points.
2. The presence of many knobs on the chromosomes is associated with primitiveness.
3. Terminal knobs are more primitive than internal knobs.
4. Changes in chromosome gradient will suffice to bring about the transition from teosinte to maize.
5. *Tripsacum* and teosinte separated early from a common ancestor.
6. Maize may have originated directly from one of many teosinte strains.

Literature Cited

1. Anderson, E. G. A chromosomal interchange in maize involving the attachment to the nucleolus. Am. Nat. **68**: 345–350. 1934.
2. ———. Chromosomal interchanges in maize. Genetics **20**: 70–83. 1935.
3. ———, and Clokey, I. W. Chromosomes involved in a series of interchanges in maize. Am. Nat. **68**: 440–445. 1934.
4. Arnason, T. J. Cytogenetics of hybrids between *Zea mays* and *Euchlaena mexicana*. Genetics **21**: 40–60. 1936.
5. Brink, R. A. The occurrence of semi-sterility in maize. Jour. Hered. **18**: 266–270. 1927.
6. ———, and Cooper, D. C. The association of semi-sterile-1 in maize with two linkage groups. Genetics **16**: 595–628. 1931.
7. ———, ———. A strain of maize homozygous for segmental interchanges involving both ends of the P-br chromosome. Proc. Nat. Acad. Sci. **18**: 441–447. 1932.
8. ———, ———. Chromosome rings in maize and *Oenothera*. Proc. Nat. Acad. Sci. **18**: 447–455. 1932.
9. Brink, R. A., and Cooper, D. C. A structural change in the chromosomes of maize leading to chain formation. Am. Nat. **66**: 310–322. 1932.
10. ———, ———. A proof that crossing over involves an exchange of segments between homologous chromosomes. Genetics **20**: 22–35. 1935.
11. Burnham, C. R. Genetical and cytological studies of semi-sterility and related phenomena in maize. Proc. Nat. Acad. Sci. **16**: 269–277. 1930.
12. ———. An interchange in maize giving low sterility and chain configurations. Proc. Nat. Acad. Sci. **18**: 434–440. 1932.
13. ———. The association of non-homologous parts in a chromosomal interchange in maize. Proc. VI Int. Congr. Genet. **2**: 19–20. 1932.

14. ————. Cytogenic studies of an interchange between chromosomes 8 and 9 in maize. Genetics **19**: 430–447. 1934.
15. Clarke, A. E., and Anderson, E. G. A chromosomal interchange in maize without ring formation. Am. Jour. Bot. **22**: 711–716. 1935.
16. Cooper, D. C., and Brink, R. A. Chromosome homology in races of maize from differing geographical regions. Am. Nat. **71**: 582–587. 1937.
17. Creighton, Harriet B. Three cases of deficiency in chromosome 9 of *Zea mays*. Proc. Nat. Acad. Sci. **20**: 111–115. 1934.
18. ————, and McClintock, Barbara. A correlation of cytological and genetical crossing-over in *Zea mays*. Proc. Nat. Acad. Sci. **17**: 492–497. 1931.
19. Fisk, Emma L. The chromosomes of *Zea mays*. Am. Jour. Bot. **14**: 53–75. 1927.
20. Kuwada, Y. Die Chromosomenzahl von *Zea mays*. Jour. Col. Sci. Imp. Univ. Tokyo **39**: 1–148. 1919.
21. Longley, A. E. Supernumerary chromosomes of *Zea mays*. Jour. Agr. Res. **35**: 769–784. 1927.
22. ————. Morphological characters of teosinte chromosomes. Jour. Agr. Res. **54**: 835–862. 1937.
23. ————. Chromosomes of maize from North American Indians. Jour. Agr. Res. **56**: 177–195. 1938.
24. ————. Knob positions on corn chromosomes. Jour. Agr. Res. **59**: 475–490. 1939.
25. ————. Knob positions on teosinte chromosomes. Jour. Agr. Res. [In press.]
26. McClintock, Barbara. Chromosome morphology in *Zea mays*. Science **69**: 629. 1929.
27. ————. A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. Proc. Nat. Acad. Sci. **16**: 791–796. 1930.
28. ————. The order of the genes, C, Sh, and Wx in *Zea mays* with reference to a cytologically known point in the chromosome. Proc. Nat. Acad. Sci. **17**: 485–491. 1931.
29. ————. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agr. Exp. Sta. Bull. 163: 1–30. 1931.
30. ————. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Zts. Zellf. Mik. Anat. **19**: 191–237. 1933.
31. ————. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Zts. Zellf. Mik. Anat. **21**: 294–328. 1934.
32. ————. The production of homologous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376. 1938.
33. ————. The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Mo. Agr. Exp. Sta. Res. Bull. 290: 1–48. 1938.
34. Mangelsdorf, P. C., and Reeves, R. G. The origin of Indian corn and its relatives. Texas Agr. Exp. Sta. Bull. 574: 1–315. 1939.
35. O'Mara, Joseph G. Cytological observations on *Zea-Euchlaena* hybrids. Genetics **24**: 82–83. 1939.
36. Randolph, L. F. Chromosome numbers in *Zea mays* 1. Cornell Univ. Agr. Exp. Sta. Memoir 117: 1–44. 1928.
37. ————. Types of supernumerary chromosomes in maize. Anat. Rec. **41**: 102. 1928.

38. Rhoades, M. M. Linkage values in an interchange complex in *Zea*. Proc. Nat. Acad. Sci. 17: 694–698. 1931.
39. ———. A cytological study of a reciprocal translocation in *Zea*. Proc. Nat. Acad. Sci. 19: 1022–1031. 1933.
40. ———. A cytogenetic study of a chromosome fragment in maize. Genetics 21: 491–502. 1936.
41. ———. On the origin of a secondary trisome through the doubling of a half-chromosome fragment. Genetics 23: 163–164. 1938.
42. ———. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics 25: 483–520. 1940.
43. ———, and McClintock, Barbara. The cytogenetics of maize. Bot. Rev. 1: 292–325. 1935.
44. Rhoades, Virginia H. The location of a gene for disease resistance in maize. Proc. Nat. Acad. Sci. 21: 243–246. 1935.
45. Stadler, L. J. On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Mo. Agr. Exp. Sta. Res. Bull. 204: 1–29. 1933.

THE BOTANICAL REVIEW

VOL. VII JUNE, 1941 No. 6

SOMATIC SEGREGATION

DONALD F. JONES

Connecticut Agricultural Experiment Station, New Haven

Plants and animals exhibiting different genotypic characters in various parts of the same individual have been described from time to time, and many hypotheses have been advanced to account for them. The first cases to be observed were mostly half and half individuals, such as insect gynandromorphs, birds with varied plumage, and fruits and flowers with stripes of different colors (27, 35, 69, 70, 103). Similar variations in somatic tissue have been observed probably in every order of the Metazoa and nearly all families of multicellular plants.

The vegetatively propagated plants, being uniform and commonly grown in large numbers, have given many examples of mosaic pattern. Some of these changes have been propagated in clonal lines as new varieties. These departures from normal generally occur after fertilization has taken place. For that reason the term "somatic or vegetative segregation" has been used (31, 99). It has been questioned fairly whether or not such a term is justified since there is no regular process by which these changes are brought about (3). They are mostly deviations from normal.

Closer examination has shown these somatic changes to occur at all stages of development and to be much more frequent than at first generally supposed. Crossing-over, occurring normally in the process of germ cell formation, also occurs in some organisms in somatic cells. The exchange of segments of non-homologous chromosomes is not essentially different from crossing-over. Both are exceptions to a normally functioning mechanism of division. Many chromosomal rearrangements, that have a visible effect only after germ cell recombination, occur before meiosis. For all of these reasons the term "somatic segregation" does seem to be useful, however these changes may be brought about. It will be used here to designate any change resulting from chromosome loss or rearrangement that is capable of propagation.

Somatic segregation has been defined by Chittenden (31) as "the production of two genetically dissimilar cells at a somatic cell division." This broader definition includes the many cases of maternal inheritance and variegation due to changes and rearrangements in the cytoplasm and the cytoplasmic inclusions. Since this evidence has been reviewed adequately (31) it will not be included here.

Chimaeras may arise from a mixture of tissues resulting from grafting (160) or from an autogenous change during development. This subject has been reviewed extensively by Baur (7), Bateson (4, 5, 6), Chittenden (31) and Jones (91). Chimaeras may result also from induced chromosomal changes (14, 55, 65, 97).

The literature dealing with somatic variations up to the present century has been reviewed carefully by Cramer (38). Since that time there are many references to "bud sports" and vegetative changes of various kinds in plants and to developmental alterations in animals, in genetic literature, especially in the Journal of Heredity. Since little critical evidence is given as to their mode of origin nothing is to be gained by listing them all here. Changes in vegetatively propagated fruits, flowers and vegetables have been tabulated recently by Shamel and Pomeroy (140), and their economic importance emphasized. Crane and Lawrence (39) have also ably reviewed the applied phases of this subject. Beijer (8) and Stout (151) list numerous changes in *Coleus*. Benedict (12, 13) gives instances of alterations in ferns, and Blakeslee (15) in non-sexually propagated fungi. East (57) summarized the somatic changes up to 1908 to show that about 75 per cent were from the dominant to the recessive condition. Reverse changes are frequent (150).

Some forms of variegation are due to infection, as pointed out by Cayley (30), Hughes (82) and Shull (141). Variations of this type have been confused with genetic changes. Fasciations, witches' brooms, galls and other abnormal growths may result from infection, injury, and also from heritable alterations (23, 80, 83, 95, 96, 102). Environmental modifications also play a part in variations that might be assumed to be germinal in origin. A remarkable case of numerous chlorophyll-free sprouts growing from the base of a normal redwood tree is reported by Cannon (26), that can not be accounted for by any obvious external or internal influences.

ATYPICAL FERTILIZATION

In 1888 Boveri (17) put forward the hypothesis of delayed or

partial fertilization to account for various insect gynandromorphs. This interpretation was based on observations in double-fertilized sea urchin eggs in which the egg nucleus divides before fertilization, one of the daughter nuclei uniting with the sperm nucleus, the other developing without fusion. In 1905 Morgan suggested an alternative hypothesis, based on the observation that more than one spermatozoon enters the egg in some cases, one of which was assumed to unite with the egg nucleus, the others developing independently without fusion. It was shown that the hypothesis of delayed and partial fertilization would account for some bilateral mosaics but not others. Polyspermy also accounts for some but not all.

In the meantime considerable attention had been given to mosaics in plants. Following the discovery by Guignard (77) and by Nawaschin (117) that the endosperm in angiosperms results from a fertilization process similar to that of the embryo, Webber (158) and Correns (37) observed cases in maize in which one half of the seed showed an immediate effect of the pollen while the other half did not. East and Hayes (58, 59) also observed many cases of this kind and called attention to their similarity to insect gynandromorphs. Following the interpretation of aberrant development in animals, various hypotheses of partial or complete lack of fusion and the subsequent independent development of haploid gametes were considered. The second male nucleus and the fused polar nuclei were assumed to develop separately to produce a mosaic endosperm. In other cases the second male nucleus was thought to unite with one polar nucleus and the other to develop independently. The evidence for this will be presented later.

CHROMOSOME ELIMINATION

For the vast majority of cases of mosaic endosperm development in maize, all of these assumptions were disproved by East (57) and by Emerson (61, 63) in finding seeds of diverse parentage in which all the cells showed characters derived from both parents while at the same time other characters were lacking in part of the cells. For example, two white-seeded types were crossed to produce purple seeds, this color requiring the complementary action of loci from both parents. One of the parents was sugary, the other starchy. Seeds resulting from the cross-pollination had all the

endosperm cells starchy, but at the same time a few seeds were found which were about one half purple and the remaining colorless. Likewise, other seeds were all purple but part starchy and part sugary. Still others were part purple and part red.

Evidence of this kind shows conclusively that fusion of germ cells has taken place and that the anomalous endosperms are due to something that takes place after normal fertilization. Emerson (63) considered further the evidence from mosaics involving the linked genes first reported by Collins (34) and showed that mutation was not the explanation for the majority of somatic changes in maize endosperm. Since more than one locus was lost simultaneously it was considered probable that chromatin elimination at some aberrant and unequal mitosis led to the production of unlike daughter cells that were viable and grew into areas of visibly different tissue.

Somewhat earlier Morgan and Bridges (113) made a detailed study of gynandromorphs and presented much carefully documented evidence from *Drosophila* to show that chromatin elimination was responsible for the majority of mosaic animals. In this material they had the advantage of using secondary sex characters as markers as well as many easily identified sex-linked genes. By using a large number of linked genes the chromosome elimination was shown to result from non-disjunction. In some cases the maternal, in others the paternal, X-chromosome was eliminated. Mosaics were also found that had resulted from the elimination of autosomes (19).

A number of special cases was listed which could not be accounted for by the theory of elimination. For these the possibility of more than one female nucleus undergoing reduction and fertilization offered a better explanation. Binucleate eggs had been observed by Doncaster (49) in *Abraxas*. Two haploid-diploid mosaics were found by Bridges (20), and L. V. Morgan (111) described three mosaics from attached-X mothers, one of which was probably due to a double nucleate egg. Sturtevant (155) added three more cases of similar derivation. Crew and Lamy (40) found forty-five female and four male mosaics in *Drosophila pseudo-obscura*. In some of these the genome derived from the male parent was present alone in a region forming a haploid area (20). In others, as the result of double nuclear fertilization, two different genomes from

the same parent were present in different areas of the body, each in combination with a genome derived from the other parent. In *Habrobracon* the majority of mosaics result from a binuclear condition (159).

A maize seed, heterozygous for genes in more than one chromosome, exhibiting all the characters of the female parent in approximately one half of the seed was found by Randolph (128) on a plant subjected to heat treatment. The other half of the seed showed characters from the male parent. Genes had to be lost from at least two chromosomes to produce this result if fertilization took place normally. The part of the seed showing only maternal characters had smaller cells than the part showing the hybrid characters. Randolph interprets this as a case of incomplete fertilization whereby one polar nucleus develops independently to produce haploid tissue, and the other fuses with the second male nucleus to produce hybrid diploid tissue having the dominant characters brought in with the pollen. This case could be due to an aberration involving two chromosomes followed by the removal of both chromosomes from the resulting cells.

Instances of irregular fertilization are comparatively rare. The vast majority of mosaics in both plants and animals are due to a change during development after fertilization has taken place normally.

SOMATIC MUTATION

Somatic mutation involving a single locus, not accompanied by any visible change in chromosome structure, has long been used as an explanation of a mosaic pattern. Collins (36) describes frequent changes from a dominant spineless condition to a recessive spiny surface on the pineapple. No alteration in the chromosomes was apparent. Mosaics attributed to mutations in animals have been described (28, 81, 101, 161). Since linked genes are not involved, the possibility of chromosomal aberrations is not excluded (41, 56). The variegated pericarp studied by Emerson (60, 62, 64), the variable foliage studied by Andersson (2), the mutable characters described by Demerec (43, 44, 45) and Imai (84), and the dotted aleurone color investigated by Rhoades (130) seem to fall in a special class and have not been accounted for by any known mechanism. Changes from colorless to colored aleurone cells in homozygous maize plants have been tabulated with a frequency of

about 1 as compared with 200 reverse changes in heterozygous plants (85). While it is impossible to test the transmission of this acquired color through germ cells, there seems to be no way that this can be accounted for on the basis of chromatin elimination.

There is always the possibility of minor rearrangements of chromatin. In somatic cells, except in especially favorable material such as the salivary glands of the Diptera, it is ordinarily not possible to detect alterations of this kind cytologically. Until more favorable material is available and better methods are known for examining the points where the alterations take place, the term "somatic mutation" is merely a label and not an explanation.

A large proportion of the mutant genes in both plants and animals are not accompanied by any visible change in the chromosomes. Some of these mutant genes are known to originate in somatic cells. Possibly many or all of them do and become visible only after germinal recombination.

DELETION

In the gynandromorphs previously noted as well as those described by Bridges (20), L. V. Morgan (111) and Sturtevant (154), the elimination of less than whole chromosomes was not mentioned. Patterson (121, 122, 123, 124) noted that the loss of parts of chromosomes, rather than whole chromosomes, played an important part in mosaic formation, and described gynandromorphs resulting from X-ray treatment that had various parts of the X-chromosome missing. A few exceptional mosaics reported by Emerson (63) indicated the elimination of a part of rather than a whole chromosome. McClintock (105, 106, 107, 109) obtained cytological evidence to prove that deletions result in color mosaics in plant tissue. Variegation results when a ring-shaped chromosome carries dominant alleles. The reduction in size or the removal of the ring from some cells, as frequently occurs, allows recessive characters to show. In hemizygous cells losses of small sections of these rings produce small areas of homozygous deficient tissue having characteristic degenerative effects. Some of these deletions are similar in their effect to the action of known recessive loci. Clausen (33) and Stino (149) report a somewhat similar mechanism for variegation in tobacco.

Stadler (145) showed that parts of chromosomes may be missing from cells of maize at the time of synapsis and that germ cells with

parts of the chromosomes missing may be viable. By following linked genes in heterozygous endosperm tissue in maize many cases of partial chromosome elimination were found (86, 143). Rhoades (131) describes a condition in which parts of chromosomes may be removed from developing tissues in maize.

PAIRED CHANGES

Much further information on the process of mosaic formation came with the discovery of twin spots in both plants and animals. Paired changes have been noted for some time in fruits and flowers. One of the first illustrations was given by Kraus (98) in apples. Later Dahlgren (42), Gibson (71) and Jones (86, 90) gave further examples. Shamel, Scott and Pomeroy (139), Frost (68) and Toxopeus (157) describe similar paired changes in citrus fruits, Lawrence (100) in *Dahlia,* and Imai (84) in *Rhododendron.* In nearly every case the change from normal is in two directions, one sector being lighter and one darker than normal in color. Eyster (66, 67) illustrated paired changes in the pericarp color of maize seeds. Similar changes were found to be common for many other characters in maize, particularly aleurone color (86). Double alterations have been reported in poultry (133) and in the rabbit (127).

Homologous chromatin exchange in somatic cells was first suggested by Serebrovsky (138), following his observations of multiple changes in the feathers of heterozygous fowls. Recessive genes appeared in mosaic areas in the same linkage relations as they were combined at the formation of the zygote. Bridges (18) first showed that hereditary material was eliminated by loss of chromatin. Belling (10) found the first cytological evidence for segmental exchange in *Stizolobium* and used this to account for the semi-sterile plants found earlier (9). Stern (146) gave the first cytological demonstration of translocation in *Drosophila.* Muller (114) found numerous chromosome rearrangements in X-rayed males of *Drosophila,* and Patterson (119, 120) demonstrated somatic rearrangement of chromosomes in *Drosophila* subjected to X-rays by obtaining twin spots. Painter (118) suggested somatic crossing-over to account for these. This possibility has been studied in detail by Stern (147) in *Drosophila,* utilizing the presence of minute bristle factors which are known to increase the number of mosaic flies (19, 156). Stern found in *Drosophila* that the mechanism of mosaic

formation was based, primarily, not upon simple elimination of chromosomes or parts of chromosomes, but on a process of somatic crossing-over involving two strands of a four-strand structure. The result is homozygosis of originally heterozygous genes without reduction in the number of chromatids. This is a mechanism for somatic segregation.

In *Drosophila*, exchange of equal parts of homologous chromosomes is facilitated by their normal association which takes place first in the prophase of the second cleavage division. It is probably significant that gynandromorphs originate during the first division before the association of homologous chromosomes takes place. Kaufmann (92, 93) found chiasmatype configurations between homologous chromosomes in somatic cells of *Drosophila*. A similar somatic pairing of homologous chromosomes in plants has been noted by several investigators (79). Peto (126) has observed chiasmata in root tips of barley. Nevertheless, there is no genetic evidence that somatic crossing-over occurs in plants or in animals outside of the *Diptera*. When specifically looked for in maize (87) no evidence was obtained for its occurrence. It was found, however, that a segmental interchange takes place, apparently at random, among all the chromosomes. Proof of reciprocal exchange among non-homologous chromosomes has been given for maize by McClintock (104, 105), Burnham (24, 25) and Rhoades (129).

Belling (10, 11) interpreted a condition of partial ovule and pollen sterility in the velvet bean, first described in 1914, as due to an exchange of non-homologous segments. Such an exchange makes each of two chromosomes deficient for a section which is replaced by a section from the other. After reduction and normal recombination, half of the gametes have one or the other modified chromosome, and, being deficient, abort. One fourth of the gametes receive both unmodified chromosomes and are unaffected. The other fourth have both modified chromosomes, but since the deficiency in one is supplied by the other, these also are functional but transmit the semi-sterile condition to succeeding generations. [For a more complete review of this evidence, see Rhoades and McClintock (132)].

Similar instances have been noted in other plants (1, 22, 24, 25, 144). They commonly result from X-ray treatment applied to the mature pollen, to young embryos shortly after fertilization, to mature

seeds and to plants at an early stage of pollen formation (143). In untreated material there is little evidence to show where the chromatin exchange actually takes place aside from the numerous cases of paired mosaics in the endosperm of the maize seed and in fruits and flowers. The stable twin spots are inferred to be reciprocal translocations from genetic evidence but have not been proved cytologically. Paired endosperm mosaics seldom occupy more than one sixteenth of the surface area of the seed. The exchange presumably occurs sometime after fertilization. In the semi-sterile ears frequently found in maize the aborted ovules usually occur over the entire structure. Presumably all the cells of the plant have chromosomes which were altered before fertilization. Rarely ears are found that are part normal and part semi-sterile. Cases of this kind indicate that the translocation occurred shortly after the ear primordial cell was laid down. Many well known pericarp color mosaics in maize indicate that changes do occur after the ear is formed, but so far there is little critical evidence to show that these changes are brought about by chromatin deletion or rearrangement. A change involving only the gonads is reported for *Drosophila* (142).

Translocations are frequent in *Drosophila,* as shown by Patterson and others (125), when X-rayed males are mated to untreated females. The exchanges involve two, three and four chromosomes. The latter two classes may result from the former by crossing-over. Most of these relocations occur in germ cells or in the immediately preceding cell generations. Stern (148) found none but homologous exchanges in untreated material in which somatic crossing-over was increased by the presence of minute bristle factors. On the other hand, Dobzhansky (47, 48) finds numerous chromosomal rearrangements in wild populations of *Drosophila.* It is not known how or where these originate. A chromosome segmental exchange in part of the cells of a salivary gland in *Drosophila* was noted by L. V. Morgan (112), and Hefler (78) found four types of tissue involving two translocations and an exchange between these in the progeny of an X-rayed male mated with an untreated female.

When maize seeds are treated with X-rays shortly after fertilization the number of endosperm mosaics is greatly increased. When these are classified according to the number of marked chromosomes involved, it is found that the number of mosaics that include

two marked chromosomes is increased proportionally more by increasing dosage than the mosaics involving only one. This indicates that the number of inter-chromosomal exchanges is increased relatively more than the number of deletions, or that translocations involving more than two chromosomes are increased over those that involve only two.

Somatic changes that involve more than two chromosomes have been observed in maize (87). Since in some cases the adjoining altered areas are approximately equal in the number of cells that they contain, the rearrangements are assumed to take place at one time in one cell or in closely succeeding cell generations. Various ways in which this may be brought about are figured by Dubinin and Khvostova (51).

CHROMOSOME BRIDGES

A novel mechanism for variegation has been described by McClintock (108) that results from cross-overs between inverted and normal chromosomes. Strands with two spindle-fiber-attachment regions are formed and these produce chromatid bridges which break at the first meiotic anaphase. Each broken chromatid behaves as if it were split, with fusions occurring between its two longitudinal halves at the position of the break. Chromosome fragments with no spindle-fiber-attachment regions may be removed from the nucleus or may pass through successive division cycles.

The bridge mechanism may be repeated in successive gametophyte cell generations and in the endosperm giving a characteristic twin-spotting variegation in color, or composition of the aleurone and endosperm cells as described by Jones (88), McClintock (110) and Clark and Copeland (32). The condition that produces the breaking may be transmitted to the endosperm through several generations. More than one chromosome bridge may be formed in the same cell, and the variegation has been seen to involve markers on at least two chromosomes. Bridges do not occur in the embryo or developing diploid tissue, according to McClintock (110). The ends of broken chromosomes appear to heal. This mechanism may account for the variegation of some fruits and flowers. The loss of fragments when the bridges are first formed may remove dominant loci, and is a means of mosaic formation with or without variegation. Similar results have been obtained by Navashin and Gerassimova (116) in *Crepis,* Müntzing (115) in rye and by Sax (135)

and Giles (72) in *Tradescantia.* Many recent investigations figure chromatin bridges.

POSITION EFFECT

When chromosomes are rearranged by inversion, duplication or translocation, unusual effects may be produced resulting from interaction in or near the regions of contact. Since these parts are normally not in close conjunction there is reason to expect an action of some kind. No doubt many recombinations of this kind are lethal and produce no visible effect. In many other cases the effect is so slight as to escape detection. Only in especially favorable material is it possible to associate alterations with chromosome relocations. When the new arrangement is altered by a return to the original pattern or a new pattern is formed, the unusual effect may disappear.

This phenomenon, called "position effect," has been demonstrated genetically by Sturtevant (152, 153) and cytologically by Bridges (21) for the bar-eye character, and by other investigators for various characters in *Drosophila* (46, 50, 51, 52, 53, 54, 73, 76, 94, 134, 136, 137). A similar result has been reported by Catcheside (29) in *Oenothera,* but has not as yet been observed in *Datura* where relocations are frequent (16). There is an indication that a position effect is shown in maize endosperm, and that this leads to cells that depart from normal in their growth relationships (89, 90). Goldschmidt (74, 75) has reviewed current theories of evolution in the light of this new evidence and concludes that the changes that result in new species are associated with a repatterning of the chromosomes.

LITERATURE CITED

1. ANDERSON, E. G. Chromosomal interchanges in maize. Genetics **20**: 70–83. 1935.
2. ANDERSSON, IRMA. Structural mosaics and inheritance of variegation in *Barbarea vulgaris.* Jour. Genet. **14**: 185–195. 1924.
3. BABCOCK, E. B., AND LLOYD, F. E. Somatic segregation. Jour. Hered. **8**: 82–89. 1917.
4. BATESON, W. Materials for the study of variation. 1894.
5. ———. Mendel's principles of heredity. 1909.
6. ———. Root-cuttings, chimaeras and "sports." Jour. Genet. **6**: 75–80. 1916.
7. BAUR, E. Pfropfbastarde, Periklinalchimären und Hyperchimären. Ber. Deut. Bot. Ges. **27**: 603–605. 1909.
8. BEIJER, J. J. Über die Knospenvariationen des *Coleus hybridus.* Genetica **14**: 279–318. 1932.
9. BELLING, J. A study of semi-sterility. Jour. Hered. **5**: 65–73. 1914.

10. ————. A unique result in certain species crosses. Zeits. Ind. Abst. Ver. 39: 286–288. 1925.
11. ————. A working hypothesis for segmental interchange between homologous chromosomes in flowering plants. Univ. Calif. Publ. Bot. 14: 283–291. 1928.
12. Benedict, R. C. The origin of new varieties of *Nephrolepis* by orthogenetic saltation. I. Progressive variations. Bull. Torrey Bot. Club 43: 207–234. 1916.
13. ————. Variation among the sporelings of a fertile sport of the Boston fern. Jour. Hered. 15: 379–394, 421–431. 1924.
14. Bergner, A. D., Avery, A. G., and Blakeslee, A. F. Chromosomal deficiencies in *Datura stramonium* induced by colchicine treatment. Am. Jour. Bot. 27: 676–683. 1940.
15. Blakeslee, A. F. Mutations in mucors. Jour. Hered. 11: 278–284. 1920.
16. ————, Avery, A. G., and Bergner, A. D. Genes associated with prime types in *Datura* and their possible relation to the hypothesis of position effect. Genetics 25: 111. 1940.
17. Boveri, Th. Über partielle Befruchtung. Sitz.-Ber. Ges. Morph. Phys. München 4: 64–72. 1888.
18. Bridges, C. B. Deficiency. Genetics 2: 445–465. 1917.
19. ————. Elimination of chromosomes due to a mutant (minute-N) in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 11: 701–706. 1925.
20. ————. Haploidy in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 11: 706–710. 1925.
21. ————. The Bar "gene," a duplication. Science 83: 210–211. 1936.
22. Brink, R. A. The occurrence of semi-sterility in maize. Jour. Hered. 18: 266–270. 1927.
23. Brown, Nellie A. Sweet pea fasciation, a form of crown gall. Phytopath. 17: 29–30. 1927.
24. Burnham, C. R. Genetical and cytological studies of semi-sterility and related phenomena in maize. Proc. Nat. Acad. Sci. 16: 269–277. 1930.
25. ————. Cytogenetic studies of an interchange between chromosomes 8 and 9 in maize. Genetics 19: 430–447. 1934.
26. Cannon, W. A. A Redwood sport. Plant World 11: 232–234. 1908.
27. Carrière, E. A. Production et fixation des variétés dans les végétaux. 1865.
28. Castle, W. E. On a transmissible tricolor variation in rats. Carnegie Inst. Publ. 320: 51–55. 1922.
29. Catcheside, D. G. A position effect in *Oenothera*. Jour. Genet. 38: 345–352. 1939.
30. Cayley, D. M. "Breaking" in tulips. Ann. Appl. Biol. 15: 529–539. 1928.
31. Chittenden, R. J. Vegetative segregation. Bib. Genet. 3: 355–439. 1927.
32. Clark, F. J., and Copeland, F. C. Chromosome aberrations in the endosperm of maize. Am. Jour. Bot. 27: 247–251. 1940.
33. Clausen, R. E. Inheritance in *Nicotiana tabacum*. X. Carmine-coral variegation. Cytologia 1: 358–368. 1930.
34. Collins, G. N. Mosaic coherence of characters in seeds of maize. U. S. Dept. Agr., Bur. Plant Ind. Cir. 132. 1913.
35. Collins, J. L. Chimeras in corn hybrids. Jour. Hered. 10: 2–10. 1919.
36. ————. A frequently mutating gene in the pineapple. Am. Nat. 70: 467–476. 1936.

37. CORRENS, C. Bastarde zwischen Maisrassen, mit besonderer Berücksichtigung der Xenien. Bibl. Bot. **53**: 1–161. 1901. [Reprinted in Carl Correns' Gesammelte Abhandlungen zur Vererbungswissenschaft aus periodischen Schriften. 1899–1924. 1924.]
38. CRAMER, P. J. S. Kritische Uebersicht der bekannten Fälle von Knospenvariationen. Naturw. Verhandl. Holl. Maatsch. Weterschappen. Haarlem 6. 1907.
39. CRANE, M. B., AND LAWRENCE, W. J. C. The genetics of garden plants. 1938.
40. CREW, F. A. E., AND LAMY, ROWENA. Mosaicism in *Drosophila pseudoobscura*. Jour. Genet. **37**: 211–228. 1939.
41. CURTIS, M. R., AND DUNNING, W. F. An independent recurrence of the blue mutation in the Norway rat and a blue-black mosaic. Jour. Hered. **31**: 219–222. 1940.
42. DAHLGREN, K. V. O. Eine Sektorialchimäre vom Apfel. Hereditas **9**: 335–342. 1927.
43. DEMEREC, M. Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. Genetics **13**: 359–388. 1928.
44. ———. Genetic factors stimulating mutability of the miniature wing character in *D. virilis*. Proc. Nat. Acad. Sci. **15**: 834–838. 1929.
45. ———. Changes in the rate of mutability of the mutable miniature gene of *D. virilis*. Proc. Nat. Acad. Sci. **15**: 870–876. 1929.
46. DOBZHANSKY, TH. Position effects of genes. Biol. Rev. **11**: 364–384. 1936.
47. ———. Genetic nature of species differences. Am. Nat. **71**: 404–420. 1937.
48. ———. Genetics and the origin of species. 1937.
49. DONCASTER, L. On the relations between chromosomes, sex-limited transmission and sex-determination in *Abraxas grossulariata*. Jour. Genet. **4**: 1–21. 1914.
50. DUBININ, N. P. A new type of position effect. Jour. Biologie **5**: 867–874. 1936.
51. ———, AND KHVOSTOVA, V. V. The mechanism of the occurrence of complex chromosome rearrangements. Jour. Biologie **4**: 935–975. 1935.
52. ———, AND SIDOROV, B. N. Relation between the effect of the gene and its position in the system. Jour. Biologie **3**: 328–331. 1934. [Also Am. Nat. **68**: 377–381. 1934.]
53. ———, AND ———. The position effect of the hairy gene. Jour. Biologie **4**: 555–568. 1935.
54. ———, SOKOLOV, N. N., AND TINIAKOV, G. G. Cytogenetic study of the position effect. Jour. Biologie **4**: 707–720. 1935.
55. DUGGAR, B. M. Biological effects of radiation. 1936.
56. DUNN, L. C. Analysis of a case of mosaicism in the house mouse. Jour. Genet. **29**: 317–326. 1934.
57. EAST, E. M. Suggestions concerning certain bud variations. Plant World **11**: 77–83. 1908.
58. ———. Xenia and the endosperm of angiosperms. Bot. Gaz. **56**: 217–224. 1913.
59. ———, AND HAYES, H. K. Inheritance in maize. Conn. Agr. Exp. Sta. Bull. 167. 1911.
60. EMERSON, R. A. The inheritance of a recurring somatic variation in variegated ears of maize. Neb. Agr. Exp. Sta. Res. Bull. 4. 1913.
61. ———. Anomalous endosperm development in maize and the problem of bud sports. Zeits. Ind. Abst. Ver. **14**: 241–259. 1915.
62. ———. Genetical studies of variegated pericarp in maize. Genetics **2**: 1–35. 1917.

63. ————. Genetic evidence of aberrant chromosome behavior in maize endosperm. Am. Jour. Bot. **8**: 411–424. 1921.
64. ————. The origin of variations. Am. Nat. **56**: 64–79. 1922.
65. Emsweller, S. L., and Brierly, P. Colchicine-induced tetraploidy in *Lilium*. Jour. Hered. **31**: 223–230. 1940.
66. Eyster, W. H. A genetic analysis of variegation. Genetics **9**: 372–404. 1924.
67. ————. Mosaic pericarp in maize. Genetics **10**: 179–196. 1925.
68. Frost, H. B. Polyembryony, heterozygosis and chimeras in *Citrus*. Hilgardia **1**: 365–402. 1926.
69. Gardner, V. R. A study of some unproductive sports of the Montmorency cherry. Jour. Agr. Res. **50**: 457–478. 1935.
70. ————, Crist, J. W., and Gibson, R. E. Somatic segregation in a sectorial chimera of the Bartlett pear. Jour. Agr. Res. **46**: 1047–1057. 1933.
71. Gibson, R. E. Chimeras and bud sports. Trans. Ill. Hort. Soc. **66**: 201–211. 1932.
72. Giles, N. Spontaneous chromosome aberrations in *Tradescantia*. Genetics **25**: 69–87. 1940.
73. Glass, H. B. A study of dominant mosaic eye-color mutants in *Drosophila melanogaster*. I. Phenotypes and loci involved. Am. Nat. **68**: 107–114. 1934.
74. Goldschmidt, R. Spontaneous chromatin rearrangements and the theory of the gene. Proc. Nat. Acad. Sci. **23**: 621–623. 1937.
75. ————. The material basis for evolution. 1940.
76. Griffen, A. B., and Stone, W. S. Gene position and mottling. Genetics **23**: 149. 1938.
77. Guignard, L. Sur les anthérozoides et la double copulation sexuelle chez les végétaux angiosperms. Rev. Gén. Bot. **11**: 129–135. [Also Comptes Rend. Acad. Sci. **128**: 869. 1899.]
78. Hefler, R. G. Two X-ray induced mosaics in *Drosophila pseudo-obscura*. Proc. Nat. Acad. Sci. **26**: 3–7. 1940.
79. Heilborn, O. The mechanics of so-called secondary association between chromosomes. Hereditas **22**: 167–188. 1936.
80. Hintikka, T. J. Inherited witches' brooms. Acta Forest. Fennica **39**: 1–15. 1933.
81. Hollander, W. F., and Cole, L. J. Somatic mosaics in the domestic pigeon. Genetics **25**: 16–40. 1940.
82. Hughes, A. W. M. Aphides as vectors of 'breaking" in tulips. Ann. Appl. Biol. **18**: 16–29. 1931.
83. Hus. H. Fasciations of known causation. Am. Nat. **42**: 81–97. 1908.
84. Imai, Y. Bud variation in a flaked strain of *Rhododendron obtusum*. Jour. Coll. Agr. Imp. Univ. Tokyo **14**: 93–98. 1937.
85. Jones, D. F. Mutation rate in somatic cells of maize. Proc. Nat. Acad. Sci. **22**: 645–648. 1936.
86. ————. Somatic segregation and its relation to atypical growth. Genetics **22**: 484–522. 1937.
87. ————. Translocation in relation to mosaic formation in maize. Proc. Nat. Acad. Sci. **24**: 208–211. 1938.
88. ————. Variegation resulting from unequal mitosis. Genetics **23**: 153–154. 1938.
89. ————. Growth changes associated with chromosome breakage and reattachment. Genetics **24**: 77. 1939.
90. ————. Nuclear changes affecting growth. Am. Jour. Bot. **27**: 149–155. 1940.
91. Jones, W. N. Plant chimaeras and graft hybrids. 1934.
92. Kaufman, B. P. Somatic mitoses of *Drosophila melanogaster*. Jour. Morph. **56**: 125–149. 1934.

93. ————. Chromosome structure in relation to the chromosome cycle. Bot. Rev. **2**: 529–553. 1936.
94. KHVOSTOVA, V. V., AND GAVRILOVA, A. A. A new method for discovering translocations. Jour. Biologie **4**: 905–916. 1935.
95. KNOX, A. A. The relation of injury to fasciation in the Evening Primroses. Plant World **10**: 145–151. 1907.
96. ————. The induction, development and heritability of fasciations. Carnegie Inst. Wash. Publ. 98: 3–20. 1908.
97. KOSTOFF, D. A chromosomal chimera in tobacco. Jour. Hered. **21**: 445–448. 1930.
98. KRAUS, E. J. Bud variations in relation to fruit markings. Or. Agr. Exp. Sta. Bien. Crop Pest & Hort. Rep. 1911–1912. 1913.
99. ————. Somatic segregation. Jour. Hered. **7**: 2–8. 1916.
100. LAWRENCE, W. J. C. The genetics and cytology of *Dahlia* species. Jour. Genet. **21**: 125–158. 1929.
101. LEVI, W. M., AND HOLLANDER, W. F. Structural anomalies and color mosaics observed in a colony of domestic pigeons. Jour. Hered. **30**: 453–457. 1939.
102. LIESE, J. Zur Bildung von Kiefern Hexenbesen. Zeits. Pilzk. **19**: 55. 1935.
103. MACARTHUR, J. W. A spontaneous tomato chimera. Jour. Hered. **19**: 331–334. 1928.
104. MCCLINTOCK, B. A cytological demonstration of the location of an interchange between non-homologous chromosomes of *Zea mays*. Proc. Nat. Acad. Sci. **16**: 791–796. 1930.
105. ————. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Mo. Agr. Exp. Sta. Bull. 163. 1931.
106. ————. A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. **18**: 677–681. 1932.
107. ————. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Zeits. Zellf. Mikr. Anat. **19**: 191–237. 1933.
108. ————. The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Mo. Agr. Exp. Sta. Res. Bull. 290. 1938.
109. ————. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376. 1938.
110. ————. The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Nat. Acad. Sci. **25**: 405–416. 1939.
111. MORGAN, L. V. Composites of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 399: 224–296. 1939.
112. ————. A spontaneous somatic exchange between non-homologous chromosomes in *Drosophila melanogaster*. Genetics **24**: 747–752. 1939.
113. MORGAN, T. H., AND BRIDGES, C. B. The origin of gynandromorphs. Carnegie Inst. Wash. Publ. 278. 1919.
114. MULLER, H. J. The production of mutations by X-rays. Proc. Nat. Acad. Sci. **14**: 714–726. 1928.
115. MÜNTZING, ARNE. Chromosomenaberrationen bei Pflanzen und ihre genetische Wirkung. Zeits. Ind. Abst. Ver. **76**: 323–350. 1939.
116. NAVASHIN, M., AND GERASSIMOVA, H. Nature and causes of mutations. 1. On the nature and importance of chromosomal mutations taking place in resting plant-embryos due to their aging. Jour. Biologie **4**: 627–634. 1935.
117. NAWASCHIN, S. Resultate einer Revision des Befruchtungsvorgangs bei *Lilium Martagon* und *Fritillaria tenelle*. Bull. Acad. Imp. Sci. St. Petersbourg 9: 1899.

118. PAINTER, T. S. A new method for the study of chromomose aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics 19: 175–188. 1934.
119. PATTERSON, J. T. X-rays and somatic mutations. Jour. Hered. 20: 260–267. 1929.
120. ———. Somatic segregation produced by X-rays in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 16: 109–111. 1930.
121. ———. Proof that the entire chromosome is not eliminated in the production of somatic variations by X-rays in *Drosophila*. Genetics 15: 141–149. 1930.
122. ———. The production of gynandromorphs in *Drosophila melanogaster* by X-rays. Jour. Exp. Zool. 60: 173–203. 1931.
123. ———. A new type of mottled-eyed *Drosophila* due to an unstable translocation. Genetics 17: 38–59. 1932.
124. ———. The mechanism of mosaic formation in *Drosophila*. Genetics 18: 32–52. 1933.
125. ———, STONE, WILSON, BEDICHEK, SARAH, AND SUCHE, META. The production of translocations in *Drosophila*. Am. Nat. 68: 359–369. 1934.
126. PETO, F. H. Association of somatic chromosomes induced by heat and chloral hydrate treatments. Canad. Jour. Res. C. 13: 301–314. 1935.
127. PICKARD, J. N. A brown-and-black rabbit. Jour. Hered. 20: 483–484. 1929.
128. RANDOLPH, L. F. Some effects of high temperature on polyploidy and other variations in maize. Proc. Nat. Acad. Sci. 18: 222–229. 1932.
129. RHOADES, M. M. A cytogenetical study of a reciprocal translocation in *Zea*. Proc. Nat. Acad. Sci. 19: 1022–1031. 1933.
130. ———. Effect of the *Dt* gene on the mutability of the a_1 allele in maize. Genetics 23: 377–397. 1938.
131. ———. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics 25: 483–520. 1940.
132. ———, AND MCCLINTOCK, B. The cytogenetics of maize. Bot. Rev. 1: 292–325. 1935.
133. ROBERTS, E., AND QUISENBERRY, J. H. A Brahma-Plymouth Rock mosaic. Jour. Hered. 26: 11–14; 114. 1935.
134. SACHAROV, V. V. The character mottled (w^{mt}) in *Drosophila* as a result of the "position effect." Jour. Biologie 5: 299–302. 1936.
135. SAX, K. An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. Genetics 25: 41–68. 1940.
136. SCHULTZ, J. Variegation in *Drosophila* and the inert chromosome regions. Proc. Nat. Acad. Sci. 22: 27–33. 1936.
137. ———, AND DOBZHANSKY, T. The relation of a dominant eye color in *Drosophila melanogaster* to the associated chromosome rearrangement. Genetics 19: 344–364. 1934.
138. SEREBROVSKY, A. S. "Somatic segregation" in the domestic fowl. Jour. Genet. 16: 33–42. 1925.
139. SHAMEL, A. D., SCOTT, L. B., AND POMEROY, C. S. Citrus fruit improvement: a study of bud variation in the Valencia orange. U. S. Dept. Agr. Bull. 624: 1–120. 1918.
140. ———, AND POMEROY, C. S. Bud mutations in horticultural crops. Jour. Hered. 27: 486–494. 1936.
141. SHULL, G. H. Variegation. Plant World 11: 147–151. 1908.
142. SIDKY, A. R. Gonosomic mosaicism involving a lethal. Jour. Genet. 39: 265–271. 1940.
143. STADLER, L. J. The experimental modification of heredity in crop plants. I. Induced chromosomal irregularities. II. Induced mutation. Sci. Agr. 11: 557–572, 645–661. 1931.
144. ———. On the genetic nature of induced mutations in plants. Proc. 6th Int. Congr. Genet. 1: 274–294. 1932.

145. ———. On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Mo. Agr. Exp. Sta. Res. Bull. 204. 1933.
146. STERN, C. Eine neue Chromosomenaberration von *Drosophila melanogaster* und ihre Bedeutung für die Theorie der linearen Anordnung der Gene. Biol. Zentralbl. **46**: 505–508. 1926.
147. ———. Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**: 625–730. 1936.
148. ———. Somatic crossing over and somatic translocation. Am. Nat. **73**: 95–96. 1939.
149. STINO, K. R. Inheritance in *Nicotiana tabacum*. XV. Carmine-white variegation. Jour. Hered. **31**: 19–24. 1940.
150. STONE, L. H. A. A somatic variation in the sweet pea. Jour. Genet. **26**: 113–114. 1932.
151. STOUT, A. B. The establishment of varieties in *Coleus* by the selection of somatic variations. Carnegie Inst. Wash. Publ. 218. 1915.
152. STURTEVANT, A. H. The effects of unequal crossing over at the bar locus in *Drosophila*. Genetics **10**: 117–147. 1925.
153. ———. A further study of so-called mutation at the bar locus in *Drosophila*. Genetics **13**: 401–409. 1928.
154. ———. The claret mutant type of *Drosophila simulans:* A Study of chromosome elimination and of cell-lineage. Zeits. Wiss. Zool. **135**: 323–356. 1929.
155. ———. Contributions to the genetics of certain chromosome anomalies in *Drosophila melanogaster*. III. Two new attached-X lines of *Drosophila melanogaster* and further data on the behavior of heterozygous attached-X's. Carnegie Inst. Wash. Publ. 421: 61–81. 1931.
156. ———. The use of mosaics in the study of the developmental effect of genes. Proc. 6th Int. Congr. Genet. **1**: 304–307. 1932.
157. TOXOPEUS, H. J. Some cases of bud-variation in *Citrus* observed in Java. Genetica **15**: 241–252. 1933.
158. WEBBER, H. J. Xenia, or the immediate effect of pollen in maize. U. S. Dept. Agr., Div. Veg. Physiol. & Path. Bull. 22: 1–44. 1900.
159. WHITING, P. W. Diploid mosaics in *Habrobracon*. Am. Nat. **66**: 75–81. 1932.
160. WINKLER, H. Über Pfropfbastarde und pflanzliche Chimären. Ber. Deut. Bot. Ges. **25**: 568–576. 1907.
161. WRIGHT, S., AND EATON, O. N. Mutational mosaic coat patterns of the guinea pig. Genetics **11**: 333–351. 1926.

ROOTROTS OF CEREALS[1]

P. M. SIMMONDS
Dominion Laboratory of Plant Pathology, Saskatoon, Canada

INTRODUCTION

Reports on observations and studies of cereal root diseases have come from many countries where grain growing is of economic importance. If the various accounts are compared, slight differences may be noted in diagnosis, prognosis and remedial measures, indicating regional influences; nevertheless, a definite unity and relationship are evident. It is helpful for purposes of research to consider the root diseases as a group. In most cereal growing areas, extensive investigations have been undertaken on these maladies. Various names have been used in designating the troubles that afflict the root system, such as foot-rot, take-all, blight, and so forth. The term rootrot, however, will be employed here to embrace broadly all diseases affecting the roots and the basal portion of the culms, and all related maladies. Most attention will be given to spring-sown cereal crops, with which the writer is most familiar. It is felt that the pathology of rice and sorghum should receive special consideration, apart from the ordinary cereals. Rather serious diseases of the roots or adjacent parts, however, of both rice and sorghum are known (23, 92, 103). The literature dealing with the early investigations on rootrots was brought together by Stevens (90). The pioneering research on cereals and flax by Bolley (10) has been of great value, especially to workers on this continent. Somewhat later, McKinney (53) reviewed the entire root disease problem. Recently, Garrett (34) published a survey on root diseases, and Bockmann (8) reviewed some of the investigations conducted in Germany. In 1939 Simmonds (80) contributed a review of the Canadian literature. The many difficulties of technique encountered in root studies have no doubt hampered investigations. The study of normal root growth under the influence of the many edaphic factors must precede or parallel pathologic studies. The importance of Weaver's (100, 101) ecological investigations and the later physiological work recently reviewed by Loehwing (49) is obvious. An attempt has been made in the present paper to

[1] Contribution No. 652, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

follow some of the trends which have appeared in root disease research, particularly during the last few years.

ROOTROT TYPES

There are several types of rootrots found in cereal crops. Some show very characteristic field symptoms and can be readily diagnosed. In other forms, a complete and satisfactory diagnosis is possible only after suitable laboratory tests. It is imperative, therefore, for the pathologist, engaged in studies upon these diseases, to become familiar with the outstanding characters of each type. He must know any special laboratory procedure which may be necessary to define doubtful cases. One may be misled by symptoms alone if first-hand observations in the field are not possible or supporting information is not available. This is a problem frequently confronting the pathologist who receives poorly prepared specimens for examination. Moreover, it is not enough to isolate an organism, even though it appears dominant, for, although suggestive, this may not give a true picture of the course of the disease in the field. The symptoms and characters of any type of rootrot are bound to be modified somewhat by climate, soil, variety and whether the grain is spring or fall sown. Investigations upon diseases of the root system are for the most part of more recent date than those dealing with diseases which affect the culm. In the latter, diagnosis has been simplified in many instances by the discreteness of the symptoms and the often intimate relationship between symptom and causal organism. This is not so evident for root diseases and necessitates a greater comprehensiveness in their diagnosis. In a review of this kind it is impossible to discuss in detail the various features of each rootrot. An effort will be made, however, to mention a few of the conspicuous characters of each type.

Take-all is a well known form of rootrot. It is caused by the fungus *Ophiobolus graminis* Sacc. It is mainly a parasite of importance on wheat, including both spring and fall sown sorts. The fungus attacks to a lesser extent barley, oats and rye. It will invade the roots, stolons and stem bases of most common grasses and may be harboured in grassland for years. In wheat crops a conspicuous field symptom is found in the patches of bleached plants which are very noticeable just prior to final ripening. Such diseased areas form a striking contrast with the surrounding healthy plants. The

heads of diseased plants contain only shrivelled kernels. The occurrence of similarly affected plants scattered here and there throughout the crop is a common but less noticeable phase of take-all. Diseased plants are easily pulled from the soil when the distinctly discoloured crown zone, crown and seminal roots indicate the principal seat of infection. These lesions, by their deep brown or black colour, and rotted and shredded tissues, are of great diagnostic value. It is always advisable, however, to complete the examination with direct microscopic observation when final proof should be obtained in the presence of the characteristic mycelium. Perithecia may be found when specimens are collected late in the season. Complete detailed accounts of this rootrot may be had elsewhere (31, 47, 53, 66). The last of these describes an inconspicuous form of the take-all rootrot which occurs in western Canada in dry years. This has been termed "dry-weather take-all" and is characterized by moderate stunting and reduced tillering. There are slight lesions on the subcrown internode and seminal roots. A microscopic examination of the lesions is necessary for definite diagnosis.

The footrot caused by *Cercosporella herpotrichoides* Fron may be considered at this point. It is not actually a rootrot but may, for our purpose, be grouped in that general category. When in severe form it is, like take-all, conspicuous in the field when the grain is in head. The two maladies, however, should not be confused (89) if sufficient attention is given to the location and character of the lesions. In the field, distinguishing signs of an attack are the location of the lesion at the base of the culms, the eye-spot lesions and the breaking-over of diseased plants. The tendency for affected plants to fall over as the heads fill, commonly results in large patches of lodged and tangled crop. The roots are not invaded to any extent. It appears that the fungus can be quite readily isolated from the lesions. *Cercosporella* footrot is mainly a disease of winter wheat. The fungus will also attack barley and rye and some grasses to a slight extent. Oats are practically immune. Glynne (37) describes the disease as it appears in winter wheat grown in England and mentions its mild attack on spring sown crops. Sprague (86), in a brief note, draws attention to the assumed relationship between *Cercosporella herpotrichoides* Fron and *Leptosphaeria herpotrichoides* de Not. The latter fungus was frequently mentioned as the cause of a stem-break or lodging disease of winter cereal in northern Europe.

A very definite root disease of wheat, which has been named browning rootrot, appears each year in western Canada. This malady has received special attention from Vanterpool (98, 96) and his students. It is caused primarily by root-infecting *Pythium* spp., particularly *P. arrhenomanes* Drechsl. and *P. tardicrescens* Vanterpool. The field characteristics are rather striking. It is most conspicuous in wheat following fallow. In Saskatchewan the onset reaches its peak early in June, when in affected crops one will notice large brown patches. A closer examination will show that the lower leaves of the seedlings are rapidly losing their green colour towards final collapse when the distinctive brown shades prevail. Although the symptoms are marked, there may be confusion with other troubles, especially a sudden wilting reaction when soil moisture is low and growth vigorous. It is necessary, therefore, to examine the seedlings for the characteristic root lesions. This is readily done by washing the roots in water while observing them against a white background. The reddish brown water-soaked lesions at the tips of the short crown roots are very noticeable. Similar lesions on the finer seminal roots are less sharply defined. A microscopic examination of the lesions to demonstrate the presence of the parasite gives final proof. The pythium lesions are found only in root tissues; rarely if ever do the invaders advance into the crown or culm base. The same *Pythium* spp. may invade the roots of other cereals and many grasses without causing much apparent harm. They have not, however, been found in the roots of dicotyledonous plants.

The very prevalent root disease caused by *Helminthosporium sativum* P. K. and B. and *Fusarium* spp. were grouped some years ago by the author under the term, common rootrot. The field symptoms and the lesions produced by the two fungi are very similar. It is conceivable that other fungi might cause similar lesions and symptoms, when the resultant disease could be classed as well in this category. The common rootrots may be found in all cereal crops and as a group present some diagnostic difficulties. In the field the attack may be noticed first during the seedling stage, when the blighted seedlings are conspicuous. Such seedlings show the usual brown and wilted lower leaves, with brown to almost black lesions at the soil surface. Although the roots bear lesions these are not conspicuous. At this stage, possible confusion with

insect damage, especially wireworms (chiefly *Ludius destructor* in western Canada) demands careful examination. Blank spaces in the drill rows indicating pre-emergence blight must be taken into account. To complete the diagnosis, it is almost always safer to wash the seedlings thoroughly and incubate them at room temperature in a moist chamber. In a few days, the fungus or fungi concerned will fructify and can be identified. From the seedling stage on, other symptoms of common rootrot may be observed. At about heading time, observations may show an unthrifty condition in certain crops or portions thereof. Frequently, the above ground symptoms are so slight as to be overlooked. This is true when a large area of the crop is affected, and contrast with nearby healthy plants can not serve as a guide. Samples taken from such areas will reveal upon examination varying degrees of lesions on the crown, basal internodes, subcrown internodes and roots. It is advisable, as mentioned above, to check such lesions by plating or incubation in order to demonstrate the invading fungi. There is, however, a conspicuous phase of common rootrot infections which appears quite widespread some seasons in western Canada. At heading time diseased plants blight prematurely, are bleached in appearance and the heads bear shrivelled grain. These bleached individuals stand out in contrast with the surrounding green plants. Examinations will show deep-seated lesions in the crown and adjacent tissues. It is possible to confuse this form of common rootrot with scattered take-all infections, so the proper laboratory procedures should follow, to complete the diagnosis. Infection due to *Helminthosporium sativum* is usually found in wheat and barley, while the *Fusarium* spp. attack oats severely and rye somewhat less so. The latter fungi also attack wheat and barley, occasionally causing severe injury, but the attack is not so consistently heavy as for the other two cereals. Common rootrot of corn, caused by *Fusarium graminearum* Schwabe (*Gibberella saubinetti* (Mont) Sacc.) and other *Fusarium* spp., is troublesome in some regions. It is particularly noticeable as a seedling blight. Further details on common rootrots may be obtained from other studies (5, 6, 19, 20, 36, 41, 43, 53, 76, 79).

A rhizoctonia rootrot of wheat and oats has been described by Hynes (44). It is caused by *Rhizoctonia solani* Kuehn. In the field the disease is usually noticed about mid-season, when one will observe patches of diseased plants. The patches vary in size, occa-

sionally extending up to three-quarters of an acre. The affected plants are distinctly stunted. They may recover somewhat as the season advances but at harvest time are characterized by a reduced number of tillers and smaller heads. The tan-coloured lesion involving the basal sheath is distinctive, and the invading hyphae can be seen with a hand lens. The main invasion, however, is in the root system. It would appear advisable, in diagnosing suspected plants, always to make a microscopic examination for the typical *Rhizoctonia* mycelium. This should be followed by isolations. The symptoms for both wheat and oats are very similar.

A rootrot of oats is caused by *Colletotrichum graminicola* (Ces.) Wils. This disease has been described and investigated by Sanford (73). The field symptoms are not particularly distinctive although affected plants are usually stunted. One should look for the rather distinct black stroma formed by the fungus on the culm near the soil surface. These have great diagnostic value, but isolations should be made especially from the seminal roots for confirmation. Boning and Wallner (11) describe a footrot of maize produced by *C. graminicola.*

The snowmold disease of winter cereals and grasses usually enters a rootrot or footrot phase as the infection develops. This has been a particularly troublesome malady in northern Europe, and its early history in that region has been mentioned by Wollenweber and Reinking (103). The fungi reported as causing the disease are *Calonectria graminicola* (Berk. and Broome) Wr. (*Fusarium nivale* (Fr.) Ces.) and *C. graminicola* var. *neglecta* Krampe (*F. nivale* var. *majus* Wr.). Other *Fusarium* spp. may also be involved in some infestations. The most characteristic field symptom is the conspicuous mold growth covering the affected seedling early in the spring. The fungus or fungi can be readily isolated, which assists greatly in diagnosis. The footrot phase which develops later may readily be confused with other rootrots, so for a complete diagnosis the early history of the onset should be known. Furthermore, it is essential to make an adequate number of platings or incubation trials with suspected lesions. The disease is fully described by Dickson (21), while a snowmold of turf grasses has been investigated and the results reported by Dahl (18).

There are, in addition, reports of snowmold in winter cereals and grasses caused by other fungi. According to Young (104), wheat

may suffer severe injury from the attacks of *Sclerotium fulvum* Fr. (*Typhula graminum* Karst.). Remsberg (63) studied the sclerotial fungi concerned in the snowmold disease. She concludes that two species of *Typhula* are involved, *T. itoana* Imai and *T. idahoensis* Remsberg. Volk (99) reports the occurrence of *T. graminum* Karst. on barley in Germany, although it seldom causes serious injury. In Russia *Sclerotinia graminearum* Elen. is associated with a similar disease of winter cereals and grasses (84).

ECONOMIC IMPORTANCE

It is difficult in most instances to determine the actual losses caused by rootrots. In severe cases quite definite estimates can be made. In milder infestations the damage done can be determined only by a careful study of numerous detailed records. This procedure is necessary for most cases of common rootrot. In estimating losses one should keep in mind not only the reduction in yield but reduction in quality. Browning rootrot, for instance, causes a retardation in ripening, exposing the wheat to frost or rust. Diseased patches in a field result in an irregular sample of threshed grain. Weed growth is encouraged when the stand is reduced by diseased roots. *Cercosporella* foot rot and common rootrots may cause lodging and so increase the difficulties of harvesting. In most studies, estimates based upon field observations and yield results are employed and of course are subject to great variations. This presents many obstacles to one who might attempt to integrate the data and draw general conclusions. Any investigator familiar with some region and who may be working on a certain root disease problem would be able to give a sound estimate of losses. Therefore, reliable estimates are available in the reports which come from the principal grain-growing areas of the world. Many such accounts dealing with particular diseases are mentioned below. There appears to have been, however, few studies dealing directly with methods of observation and computation in regard to losses caused by root diseases. The only compilations available were made on this continent. In connection with the plant disease survey of the United States, records are compiled and estimates made each year on crop losses.

A fair notion of the economic importance of rootrots as a group may be obtained from recent compilations (24, 25). The computa-

tion methods have been carefully devised although the original figures are based on estimates. The methods are explained in the first reference mentioned. It is important to remember that the loss is based on the possible yield, had the crop been free of disease. In wheat, the losses varied from a trace to around 10% of the estimated possible yield. For barley and rye, the losses were much the same, while oats and corn showed greater injury. A large amount of grain is involved in such losses, especially in the cereal-growing states. For wheat in Minnesota in 1938, a 3% reduction indicated a loss of 1,425,000 bushels. Barley showed a reduction of 1% or 493,000 bushels, corn 1% or 1,676,000 bushels, while oats showed only a trace. Many states had larger losses for some crops while others report only a trace. It is of interest that for the same year in Minnesota losses from wheat scab (head-blight) was 7% or 3,325,000 bushels. The records for rootrots, when compared with other maladies listed, indicate that they must be considered of major importance along with the rusts, smuts and so forth. Losses caused by cereal root diseases, particularly common rootrots in western Canada, were studied by Greaney (40). Field experiments conducted over a period of years showed that yield was inversely proportional to the degree of common rootrot infection. In 1939 field surveys were made and a large number of wheat plant samples were collected. The plants were classified according to the severity of the lesions on the subcrown internode. The yield from each class and its relationship to infection or freedom from disease was recorded. The percentage reduction in yield for each class was readily determined and could be applied in interpreting the entire survey records for the region. With information thus obtained from field experiments and surveys, it was possible to compute the average annual loss from records obtained over the period 1930 to 1939. The average annual loss in wheat in Manitoba was placed at 5% or 1,969,345 bushels and in Alberta 3% or 3,609,420 bushels. In Saskatchewan the actual records worked out to 9.4% which was considered high, judging by recent studies of similar data. If 5% is taken for this province, the loss in bushels would be 6,860,000. It was mentioned by Greaney, however, that such figures must be considered as approximations. It is difficult to predict with finality how much or how little injury a certain degree of infection may cause. The above losses for western Canada refer

only to common rootrot. Detailed records are not available for browning rootrot, but some seasons the losses are very heavy, probably exceeding the above figures. Take-all has not been widespread lately but it is known to be a very destructive disease under suitable conditions. Further studies on methods of recording and computing losses by rootrots is needed. Severe outbreaks resulting in heavy losses are frequently confined to local areas, perhaps involving only a few farms. Such cases can usually be traced to some particular practice of cultivation or to the use of poor or injured seed or both. These losses are very real for the farmer or district concerned. The tendency for cereal yields to decrease in older farming regions, unless proper precautions are taken to maintain soil fertility, has been generally recognized. The probable part played by root diseases in this decline has not been adequately studied.

RESEARCH TRENDS AND RESULTS

Take-all rootrot soon came to the attention of farmers and agricultural scientists because of its conspicuous field symptoms. The causal fungus, *Ophiobolus graminis* Sacc., has interesting characteristics and has received much attention from both mycologists and pathologists. Fitzpatrick, Thomas and Kirby (29) considered the correct name to be *O. cariceti* (B. and Br.) Sacc. but this was questioned by McKinney (53). The nomenclature problem was reviewed by Russell (66) who found some evidence in support of *O. cariceti* but, like most workers, felt the more familiar name of *O. graminis* should be maintained for the present. Pathogenicity tests were successfully conducted by the earlier investigators and confirmed by recent studies. The mode of infection and the course of the disease were followed in detail by Fellows (27) and Robertson (64). The fungus may enter by way of the roots, subcrown internode or coleoptile. It may enter the crown as the invasion advances from the subcrown internode or crown roots. The fungus penetrates the epidermis readily and soon becomes established in parenchyma tissues. Virulent strains of *O. graminis* make rapid progress within the host under favourable conditions. Studies of numerous isolates, however, show that they vary widely, not only in pathogenicity but also in cultural and other characters. An isolate kept in culture for some time may show distinct fluctuations in virulence.

Studies upon the questions of virulence and possible biologic strains, which is of great importance for plant breeding purposes, have been reported by Bussmann (13), Padwick (59) and Russell (67). The virulence and pathogenic behaviour of *O. graminis* are greatly influenced by other organisms, as was demonstrated in the investigations conducted by Sanford and Broadfoot (75) and Henry (42). These findings opened up an interesting approach and stimulated much similar research in cereal root pathology. Brommelhues (12), working in Germany, concluded from her tests that *O. graminis* is inhibited in associative cultures by toxins secreted from certain fungi. Furthermore, in soil trials similar toxins were found to affect wheat seedlings when previously exposed to their action, in such a way as to make them more susceptible to the attacks of the take-all fungus. Under ordinary field conditions, it was suggested, the toxins are adsorbed in clay soil mixtures but are free to act in sandy soil, where observations have shown *O. graminis* to be very virulent. The dependence of *O. graminis* upon certain growth factors for its full and vigorous development has been suggested by Padwick (60). He believes that these may be as important as toxins or nutrient deficiencies in affecting the virulence of this pathogen.

Some of the earlier work can be re-interpreted in the light of new information. As early as 1927 Russell (65) had commenced investigations to determine the longevity of *O. graminis* in fallow soil. This work was completed and discussed in a later publication (66). It was found that the fungus remained alive in fallow soil in pots for two years outside and for one year in the greenhouse. It appears that the difference here can be explained on the basis of the lower temperature outside suppressing the activity of other soil microorganisms. Garrett (33) has shown that the resting mycelium of *O. graminis* in soil rapidly disappears when conditions are favourable for microbiological activity. Working in England, also, Lal (48) found that the fungus persisted for over five months in sandy alkaline soils but disappeared in a few weeks in acid soils. The greater number of soil organisms, particularly antagonistic fungi, in the acid soils were held responsible for the decline. The outbreaks of take-all are greatly restricted when the causal fungus is deprived of suitable hosts for a period of time. It was observed by Padwick (58) that the fungus spreads along the living roots of

susceptible plants. This characteristic of *O. graminis* was clearly demonstrated in experiments conducted by Garrett (30). Recently, Fellows and Ficke (28) studied the infestation and spread of *O. graminis* and reviewed the literature on this problem. Their results confirm the findings of Garrett in respect to the importance of living roots for the spread and maintenance of the fungus. They mentioned the possibility of soil particles and plant debris playing a part in dissemination but consider them insignificant. It was found that ascospores may serve to spread the fungus but are probably unimportant under field conditions, which is in accord with the findings of Garrett (35) and Padwick (61). In compacted soil where aeration is restricted, infections by the take-all fungus may be greatly reduced, according to experiments and observations reported by Winter (102). This is in agreement with Garrett's (32) hypothesis respecting the importance of aeration to the progress of the fungus during its parasitic phase, especially in acid soils. It will be observed that the trend of researches on this disease has been towards a better understanding of the causal fungus, its life-history, its persistence and existence in the ever-changing soil habitat. The earlier workers knew the importance of crop rotations and cultural practices in the control of take-all. Recent work has secured and broadened the foundations upon which the early recommendations were made. Generally speaking, when take-all appears in a crop of wheat in severe form, the rotation must be modified to bring in a fallow year or employ a non-susceptible crop. Of the ordinary cereal crops, oats is the only one which can be considered highly resistant and safe to use. Corn is not affected. All the dicotyledonous field crops can be considered as practically immune. None of the wheat varieties tested by various workers shows any appreciable resistance. The fungus attacks barley and rye and most grasses. The significance of grasses in the maintenance and increase of natural inoculum is well known. Recent accounts dealing with host range and general control methods will be found in other papers (1, 31, 56, 66, 72).

Investigations on the common rootrot complex of cereals confronts the pathologist with many difficulties. Unlike take-all and some other root diseases, the symptomatology and etiology of this group are subject to great variability. The problems encountered are much the same, whether one deals with the group as a whole,

as we shall do here, or with any component thereof. For many years the attacks may be mild but prevalent, resulting in general debility which is not noticed by the layman. Then under suitable conditions the disease appears in severe form, occasionally in small areas, reaching almost epidemic proportions. Such outbreaks were bound to bring the malady to the attention of both farmer and pathologist. The early investigators readily isolated numerous fungi from diseased plants and conducted pathogenicity tests. It was shown that *Helminthosporium sativum* which was commonly isolated from diseased wheat plants could also attack barley, rye and some grasses. Although *H. sativum* appears to be by far the most important, nevertheless, there are related species of *Helminthosporium* and *Curvularia* involved in similar root diseases, as outlined by Hynes (45).

The next important group of fungi to be constantly associated with diseased plants was *Fusarium* spp. Seedling blight and rootrot of corn, wheat, barley, oats and rye and many grasses may be caused by these fungi, notably *F. graminearum* Schwabe, *F. culmorum* (Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc. Other species of *Fusarium* and other fungi have occasionally been isolated from cereal plants showing common rootrot. Most of these have proved to be of little importance. Because of the tendency for both *Fusarium* spp. and *Helminthosporium sativum* to show great variability in culture, studies were soon undertaken in an attempt to determine its significance. Thus both Christensen (14) and Hynes (43) showed that there were numerous physiologic forms of *H. sativum.* The forms differed in their pathogenicity as well as in many other respects. According to studies by Eide (22) with isolates of *Gibberella saubinetti* (Mont.) Sacc. (*F. graminearum* Schwabe), differences in virulence were found in the cultural variants. Pathogenicity tests were made on corn and wheat. Pathologists working with *Fusarium* spp. were confronted not only with great variations within a species but also with perplexing taxonomic problems. Some phases of the latter difficulties, as they pertain to cereal pathology, were studied by Bennett (2, 3, 4) and Gordon (38) with most valuable results. Satisfactory progress in such studies was made possible through the comprehensive taxonomic studies on this genus pursued by Wollenweber and his colleagues, culminating in the recent book by Wollenweber and Reinking

(103). It was quite necessary to understand the extent and degree of fluctuations in pathogenicity in order to develop a sound pathological approach in any plant breeding program. Rainio (62) tested a large number of oat varieties against *Gibberella saubinetii* (Mont.) Sacc. and found certain varieties to be more resistant than others. The differences were explained largely on the basis of morphological characters of the hosts. Extensive experiments were conducted by Greaney *et al.* (39). They found that some varieties of spring wheat were more resistant to attacks of *H. sativum* and *F. culmorum* than others. Moreover, their tests with oat varieties indicated differences in resistance to *F. culmorum*. In this work it was observed that many of the newly developed rust-resistant varieties of both wheat and oats were likewise resistant to common rootrot. This may indicate a relationship between vigor and rootrot resistance, for careful field trials are always conducted by the plant breeder in selecting his material for yield and adaptability to the region. Needless to say, all susceptible strains would be exposed to the ubiquitous common rootrot fungi, and diseased and unthrifty material would be discarded in most cases. One of the obstacles, especially in artificial inoculation experiments, was the inconsistency of pathogenicity reactions. This was true for tests to determine varietal resistance as well as in other experiments especially under field conditions or when unsterilized soil was employed. It was, of course, later shown that these irregularities were related to antibiosis or similar phenomena, comparable to those encountered in work with *O. graminis*. Sanford (74) recently summarized the pathological aspects of this problem. A further contribution from the same laboratory by Sanford and Cormack (77) throws new light on the problem in regard to the virulence of *H. sativum*. They demonstrated, chiefly with isolates of *Penicillium*, that the association effect may differ for random isolates, and therefore it is not sufficient to merely consider the species or genus in such studies. The poor results obtained in early investigations with mash or other bulk inocula could in most cases be explained by the action of soil microorganisms. To surmount this and other defects, Sallans (69) improved the spore suspension procedure for inoculations which gave good results with both *Helminthosporium sativum* and *Fusarium culmorum*. Disease development may be greatly influenced by soil temperature and soil moisture, as shown by the work of

Dickson (20), McKinney (52), Hynes (46) and others. In general, the fungi concerned will infect the roots and crowns, causing disease manifestations over a wide range of soil temperature and moisture. These factors are constantly changing under field conditions, making interpretations of the involved pathology very difficult. It is generally recognized on this continent, however, that infections and injuries are reduced when spring grain is sown early, and fall grain sown late, to provide cool soil temperatures through the seedling stage. Sallans (70) conducted greenhouse tests on the use of water by healthy wheat plants and ones inoculated with *H. sativum*. Although the diseased plants tended to recover from early infections, it was suggested that under field conditions with restricted moisture and weed competition recovery would be uncertain. In later work, Sallans (71) showed that weed growth developed freely around diseased plants, preventing complete recovery and resulting in reduced yields. In looking back over the work at this juncture, one can not see major and distinct trends which may soon lead to suitable control measures. This is in contrast to the investigations on take-all where feasible control methods were quickly brought to light and by subsequent research, improved and confirmed. The results to date, however, are encouraging when the magnitude and complexity of the problem are kept in mind. Most workers are agreed that good farming practices which promote strong and vigorous growth are helpful in reducing losses from common rootrots. New and useful approaches may be expected from the analytical and exploratory work now underway. A very complete review of the control problem was made recently by Hynes (46), particularly in regard to the *H. sativum* type of common rootrot.

Investigations on *Cercosporella* footrot of wheat, barley and rye have been conducted in the United States and in northern Europe. In Europe it was known as the stem-breaking disease and was believed to be caused by *Leptosphaeria herpotrichoides* De Not. It frequently caused severe damage, particularly in fall sown cereals (26). There is no doubt the most important contribution in regard to this footrot was the demonstration by Sprague (85) that *Cercosporella herpotrichoides* Fron was the true causal fungus. Further studies by Sprague (86) and Sprague and Fellows (89) confirmed the active parasitic nature of *C. herpotrichoides*, whereas,

in comparison, *Leptosphaeria herpotrichoides* was at best a weak parasite. There soon followed supporting evidence from Europe and England indicating that attacks by *C. herpotrichoides* was the cause of stembreak and lodging in cereals (37, 57, 78). Various attempts had been made to develop methods of control, even before the etiology was fully understood. A black fallow in the crop rotation may be used to "starve out" the parasite, according to Meyer-Bahlburg and Bindfeil (55). This, however, is considered to be uneconomical, and as an alternative method they suggest deep ploughing to bury the stubble of the preceding crop. The latter practice gave good results. Glynne (37) mentions the probable reduction in primary infections as a result of a fallow year in the rotation. The crop following the fallow, however, would be heavier as a result of an increase in available nitrogen and might consequently be predisposed to severe secondary infections. Long rotations with wheat and barley widely separated should help in reducing injury from this rootrot. Sprague and Fellows (89) recommend crop rotations and suggest oats, alfalfa and other leguminous crops which may be grown safely in infested areas. Tests to determine the susceptibility of cereals and grasses were conducted by Sprague (87). He found no true resistance in the cereals although oats, einkorn, winter rye and late-maturing wheats occasionally escape injury. In the grasses, species of *Agropyron* were quite susceptible while species of *Festuca* were resistant. Some control of *Cercosporella* footrot may be obtained in winter wheats, according to Bockmann (9), by thin, shallow and late seeding. The first-mentioned factor is the most important, for thick heavy crops frequently showed severe injury. Mention should be given to the extensive studies (88) on the influence of climatological factors on the development of this rootrot. It is one of the few studies of its kind in this field and no doubt will stimulate further interest in phenological observations. The short period of time in which this disease has been under investigation in the United States has led to a clear understanding of its etiology which in turn points the way towards satisfactory control practices.

Browning rootrot has been of great economic importance in western Canada. It is primarily a disease of wheat in the area where the short, wheat-wheat-fallow rotation is employed. It appears in severe form in the crop following the fallow year. This

presents a real problem in the semi-arid cereal regions where fallowing is important in the conservation of moisture and weed control. *Pythium* spp., including those found to be pathogenic on cereals, are widespread (95). One might expect, therefore, the same or a similar malady to appear in cereal-growing regions where a short rotation including a fallow period is practised. The disease may be readily overlooked or confused with other troubles, as mentioned above in describing the field symptoms. Preliminary research on the problem in western Canada indicated that it should receive serious study. The results of investigations over a ten-year period have been recently summarized (96). The mycological and nutritional factors concerned in the etiology were analyzed and studied, bringing to light fundamental facts upon which remedial measures could be based. Field observations, greenhouse tests, soil analyses and fertilizer experiments soon led to a satisfactory explanation of the pathology involved. It was shown that unbalanced nutrition, particularly the available phosphorus-nitrate relationship, predisposed the wheat seedlings to attack by *Pythium* spp. Fluctuations in the available phosphorus were of greatest importance. In districts where browning rootrot persists year after year, applications of phosphatic fertilizers, especially ammonium phosphate, to the crop on fallowed land almost invariably prevents serious injury. In addition, all other means of assuring good soil fertility and a vigorous crop must be continued. The investigations failed to show sufficient disease resistance in any of the ordinary Canadian wheats to be of importance as a means of control. The appearance of the rootrot from year to year and district to district is quite unpredictable, although it can be expected frequently where the soil is deficient in phosphorus. It would, therefore, be of the greatest value if future studies should reveal ways and means of foretelling its probable occurrence. Then the fertilizer corrective could be employed with assurance. Vanterpool and Simmonds (97) have shown that crops delayed by browning rootrot attack tend to show a heavier infection of stem rust. This is just one instance of many where root troubles may influence the development of diseases of the shoot. Van Luijk (94) observed that some saprophytic soil fungi stimulated the growth and intensified the attack of grasses by certain *Pythium* spp.

The remaining types of root diseases described above may be

discussed briefly. They are not necessarily of lesser importance, even though studies and the information available concerning them are limited in scope. *Rhizoctonia* rootrot of wheat and oats has a history somewhat similar to that of browning rootrot. Species of *Rhizoctonia* are common soil fungi of world-wide distribution. Presumably, ecological factors were favourable in the Australian cereal-growing regions to the predisposition of the hosts and the active parasitism by the fungus. Consequently, severe outbreaks of the disease have been reported only from Australia, as browning rootrot has received attention only in Saskatchewan, Canada. *Rhizoctonia* rootrot has been investigated by Hynes (44) who also reviews the earlier observations and studies. It was concluded from the results of these investigations that the only practicable means of combatting the malady is in the application of sulphate of ammonia fertilizer. The fertilizer stimulated the host and possibly also inhibited the parasite to some extent. Oats responded to control attempts much better than wheat.

Turning now to the snowmold complex, one can see that these troubles, although confined to winter cereals, are serious problems for certain areas. Losses are frequently great (104). Recent studies (63) towards clarifying the taxonomic position of the sclerotial fungi found associated with snowmolds are fundamental to this problem. Contributions from Russia (84, 93) and from Germany (99) add much needed information to the study. No specific control recommendations are available, although the Russian workers state that some varieties of winter wheat show resistance to the attack of *Sclerotinia graminearum* Elen. Crop rotation, dates of seeding and the use of fertilizers, modified in accordance with the regions concerned, have been mentioned as means for reducing injury from snowmolds.

The development of diseased and healthy roots of wheat has been studied by Simmonds and co-workers (83). The data obtained in these investigations were used in interpreting reactions and injuries caused by rootrots (81). A wheat plant produces a seminal or first root system and later a crown root system. Root amputation experiments showed that injuries to the seminal roots may be very serious during early stages of growth. Similarly, infections by rootrot fungi of the first roots may cause seedling blight or a stunted and delayed development if the plant tends to recover. Am-

putations of the crown roots later in the season may also result in severe injury. This is somewhat comparable to deep crown and crown root infections which commonly causes bleaching and blighting at heading time.

RELATED PROBLEMS

In root disease studies one must keep in mind the fact that some of the root-invading fungi will attack other parts of the plant. Therefore, in addition to immature and shrivelled seed as a result of root and crown invasion, the matter of infected seed must be appraised. This latter point is of interest from both the seeding and feeding angles. Also the general health status and vigor of seed samples have direct bearing on the initiation of root invasion, especially during the seedling stage. The question of seed-borne pathogens has been investigated by many workers in conjunction with root disease studies. Infected seed is usually discoloured, but discoloration alone is not sufficient upon which to make recommendations or draw conclusions. Attempts should be made to determine the presence of pathogenic fungi by isolation or incubation methods. The common rootrot fungus, *Helminthosporium sativum*, causes a leaf spot, head blight and kernel discoloration in wheat, barley and rye. While *Fusarium* spp., particularly *F. graminearum*, *F. culmorum* or *F. avenaceum*, may cause head blight in wheat, barley, rye and oats. Infected wheat and barley kernels result in seedling blight and an increase in rootrot if used for seed (15, 17, 51). Treatment with an organic mercury dust reduced the injury. Blair (7) obtained beneficial results in inoculation tests when he employed the mercury dust treatments. He observed that the fungicide protected the seedling only through the early stages. Severe infections may occur later in the plant's development. Machacek and Greaney (50) and Mead (54) have shown that various forms of seed injury predispose the seedlings to an attack by rootrot fungi. The examination of cereal seed samples, as a routine practice, for the determination of the disease factor has been proposed by Simmonds and Mead (82). They maintain that rootrot fungi can be readily detected if the proper technique is applied. Further studies on cereal seed examinations conducted in this laboratory have been completed and submitted for publication (68). A few years ago, the relationship between blighted or scabby barley and poisoning of livestock, especially pigs, caused much concern. This stimulated

research on the toxicity of fungus-infected food. Extensive tests with blighted barley were conducted by Christensen and Kernkamp (16). They studied numerous fungi and found *F. graminearum* to be the most active in the production of a toxic substance. It also produced a toxin when growing on wheat and corn. The importance of *Fusarium* spp. in toxicity studies with fungi is shown by the experiments of Steyn (91) who reviews the entire problem.

CONCLUSIONS

In surveying some of the problems in rootrot studies one is impressed with the distinct difference between these diseases and other cereal maladies, such as the rusts or smuts. The explanation is found in the close association of rootrots with the soil and soil conditions. The pathogens must be well established as a rule in the soil or on crop debris before becoming a potential source of infection. It is not likely, therefore, that climatological factors have a direct bearing upon root disease epiphytotics, such as wind direction and velocity have in the dissemination of the rusts. An exception, however, may be noted in the case of snowmolds, where the time of snow-fall and its extent are very important. The indirect influence of weather and climate in modifying the soil can not be overlooked. Rainfall, drying winds and soil temperature may be decisive factors in determining an outbreak of most root diseases. Of equal and probably greater importance are methods of cultivation, crops grown, and general farm practices peculiar to certain regions. When these are favourable to the development of a disease type, it will assume dominance. A change in the system of agriculture might submerge one type only to bring another to the fore. For instance, in western Canada, the fallow year is not conducive to the development of take-all, but, on the other hand, it promotes infestation by browning rootrot. Take-all incidence is well known to be closely linked with rotations including grasses. It is particularly severe in regions where grasses must be maintained in the rotation for sound economic reasons. In districts where winter cereals are grown, a set of conditions arises favouring outbreaks of *Cercosporella* footrot and snowmold. There are many similar cases all of which point towards the intimate connection between root disease problems and any agricultural production project. The introduction of new varieties or changes in methods of cultivation may

confront the pathologist with an entirely different array of problems. Although the regional influences must be kept foremost in any research program, nevertheless, efforts should be equally directed towards defining the various types of root disease. It is not sufficient to deal only with the dominant type, but all associated infestations must be analyzed and their significance appraised.

Recent investigations have led almost invariably towards a greater exploration of the soil relationship. The series of papers which have appeared dealing with antibiosis and related phenomena have cleared up many troublesome points. Yet, perplexing problems remain. It is certain, however, that the present emphasis on the soil microbiological aspects is a sound and fundamental approach. The physical state of the soil should be kept in mind, as shown by Winter's (102) investigations. The ordinary agricultural practices of cultivation, fertilizer treatments, and so forth, all alter the physical state of the soil. Such changes affect the microbiological activity and in turn may change the disease prospect. It is important, therefore, that more data be accumulated on the influence of soil treatments, for such lie within the limits of practicable control measures. This aspect and the influence of crop sequence offer the most promising approach towards a feasible remedy for many root maladies. A comparative study of the growth of healthy and diseased roots under different soil conditions has revealed some points of interest. It is known that varieties may have different rooting habits. The vigor of the crown and seminal root development in wheat may have considerable bearing on determining the course and result of a root infection. Physiological aspects of root diseases, which are now receiving some attention, would appear to be a fruitful line for research. One would expect a severe root infection to greatly alter the absorbing qualities of these important organs.

Literature Cited

1. Akerman, A., Granhall, I., and Hagander, H. Undersökningar över olika förfrukters inverkan a Veteskorden, särskilt med hänsyn till angrep av rotdödare (*Ophiobolus graminis*). Sverig. Utsädesforen. Tidskr. **44**: 386–408. 1934. [German summary.]
2. Bennett, F. T. On two species of *Fusarium, F. culmorum* (W. G. Sm.) and *F. avenaceum* (Fries.) Sacc., as parasites of cereals. Ann. App. Biol. **15**: 213–244. 1928.
3. ———. *Fusarium* species on British cereals. Ann. App. Biol. **20**: 272–290. 1933.
4. ———. *Fusarium* species on British cereals. Ann. App. Biol. **22**: 479–507. 1935.

5. ————. *Fusarium* disease of cereals. Agr. Prog. **16**: 1–6. 1939.
6. Blair, I. D. An investigation of footrot of wheat in New Zealand. N. Z. Jour. Sci. Tech. **19**: 1–21. 1937.
7. ————. Seed disinfection trials with cereals. N. Z. Jour. Sci. Tech. **20**: 128–132. 1938.
8. Bockmann, H. Die Getreidefusskrankheiten. Forschungsdienst. **2**: 68–71. 1936.
9. ————. Möglichkeiten zur Verhütung der Halmbruchkrankheit bei Getreide. Pflanzenbau **15**: 403–430. 1939.
10. Bolley, H. L. Soil troubles and seed deterioration. Causes of soil sickness in wheat land. Possible methods of control and cropping methods with wheat. N. D. Agr. Exp. Sta. Bull. 107. 1913.
11. Böning, K., and Wallner, F. Welke. Fusskrankheit und andere Schädigungen an Mais durch *Colletotrichum graminicolum* (Ces.) Wilson. Phytopath. Zeit. **9**: 99–110. 1936.
12. Brömmelhues, Maria. Die wechselseitige Beeinflussung von Pilzen und die Bedeutung der Pilzkonkurrenz für das Ausmass der Schädigung an Weizen durch *Ophiobolus graminis* Sacc. Zbl. Bakt., Abt. 2. **92**: 81–116. 1935.
13. Bussmann, B. Untersuchungen über die Virulenz von *Ophiobolus graminis* Sacc. Phytopath. Zeit. **9**: 571–581. 1936.
14. Christensen, J. J. Physiologic specialization and parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bull. 37. 1926.
15. ————. Associations of microorganisms in relation to seedling injury arising from infected seed. Phytopath. **26**: 1091–1105. 1936.
16. ————, and Kernkamp, H. C. H. Studies on toxicity of blighted barley to swine. Minn. Agr. Exp. Sta. Tech. Bull. 113. 1936.
17. ————, and Stakman, E. C. Relation of *Fusarium* and *Helminthosporium* in barley seed to seedling blight and yield. Phytopath. **25**: 309–327. 1935.
18. Dahl, A. S. Snowmold of turf grasses as caused by *Fusarium nivale*. Phytopath. **24**: 197–214. 1934.
19. De Haan, J. T. Untersuchungen über das Auftreten der Keimlingsfusariose bei Gerste, Hafer, Mais and Reis. Phytopath. Zeit. **10**: 235–305. 1937.
20. Dickson, J. G. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetti*. Jour. Agr. Res. **23**: 837–870. 1923.
21. ————. Diseases of cereals and forage crop plants. 1939.
22. Eide, C. J. The pathogenicity and genetics of *Gibberella saubinetti* (Mont.) Sacc. Minn. Agr. Exp. Sta. Tech. Bull. 106. 1935.
23. Elliott, C., Melchers, L. E., Lefebvre, C. L., and Wagner, F. A. *Pythium* rootrot of milo. Jour. Agr. Res. **54**: 797–834. 1937.
24. Edson, H. A., Wood, Jessie I., and Nance, Nellie W. Estimates of crop losses from diseases in the United States in 1935. U. S. Dept. Agr. Bur. Pl. Ind. Pl. Dis. Rep., Suppl. 94. 1935.
25. Edson, H. A., and Wood, Jessie I. Crop losses from plant diseases in the United States in 1938. U. S. Dept. Agr. Bur. Pl. Ind. Pl. Dis. Rep., Suppl. 118. 1939.
26. Eriksson, J. Fungoid diseases of agricultural plants. 1912.
27. Fellows, H. Some chemical and morphological phenomena attending infection of the wheat plant by *Ophiobolus graminis*. Jour. Agr. Res. **37**: 647–662. 1928.
28. ————, and Ficke, C. H. Soil infestation by *Ophiobolus graminis* and its spread. Jour. Agr. Res. **58**: 505–519. 1939.
29. Fitzpatrick, H. M., Thomas, H. E., and Kirby, R. S. The *Ophiobolus* causing take-all of wheat. Mycologia **14**: 30–37. 1922.
30. Garrett, S. D. Soil conditions and the take-all disease of wheat. Ann. App. Biol. **23**: 667–699. 1936.

31. ————. Take-all or whiteheads disease of wheat and barley and its control. Jour. Roy. Agr. Soc. **98**: 24–34. 1937.
32. ————. Soil conditions and the take-all disease of wheat. II. The relation between soil reaction and soil aeration. Ann. App. Biol. **24**: 747–751. 1937.
33. ————. Soil conditions and the take-all disease of wheat. III. Decomposition of the resting mycelium of *Ophiobolus graminis* in infected wheat stubble buried in the soil. Ann. App. Biol. **25**: 742–766. 1938.
34. ————. Soil-borne fungi and the control of root disease. Imp. Bur. Soil Sci., England, Tech. Comm. 38. 1939.
35. ————. Soil conditions and the take-all disease of wheat. IV. Factors limiting infection by ascospores of *Ophiobolus graminis*. Ann. App. Biol. **26**: 47–55. 1939.
36. ————, Glynne, Mary D., Hickman, C. J., Williams, P. H., and Ogilvie, L. Symposium and discussion on rootrots. Trans. Brit. Myc. Soc. **23**: 209–213. 1939.
37. Glynne, Mary D. III. Eyespot lodging of wheat caused by *Cercosporella herpotrichoides* Fron. Agr. Prog. **16**. 1939.
38. Gordon, W. L. Species of *Fusarium* isolated from field crops in Manitoba. Proc. World's Grain Exhib. & Conf., Regina, Canada **2**: 298–299. 1933.
39. Greaney, F. J., Machacek, J. E., and Johnston, C. L. Varietal resistance of wheat and oats to rootrots caused by *Fusarium culmorum* and *Helminthosporium sativum*. Sci. Agr. **18**: 500–523. 1938.
40. ————. Losses caused by root diseases of cereals in western Canada. (Unpublished data, 1940.)
41. Henry, A. W. Rootrots of wheat. Minn. Agr. Exp. Sta. Tech. Bull. 22. 1924.
42. ————. Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis*. Canad. Jour. Res. **7**: 198–203. 1932.
43. Hynes, H. J. Studies on Helminthosporium rootrot of wheat and other cereals. Part 1. Economic importance and causal organisms. Part 2. Physiologic specialization in *Helminthosporium* spp. N. S. W. Dept. Agr. Sci. Bull. 47. 1935.
44. ————. Studies on rhizoctonia rootrot of wheat and oats. N. S. W. Dept. Agr. Sci. Bull. 58. 1937.
45. ————. Species of *Helminthosporium* and *Curvularia* associated with rootrots of wheat and other graminaceous plants. Jour. Roy. Soc. N. S. W. **70**: 378–391. 1937.
46. ————. Studies on *Helminthosporium* rootrot of wheat and other cereals. Part 3. Factors influencing infection. Part 4. The control problem. N. S. W. Dept. Agr. Sci. Bull. 61. 1938.
47. Kirby, R. S. The take-all disease of cereals and grasses caused by *Ophiobolus cariceti* (Berk. & Br.) Sacc. Cornell Univ. Mem. 88. 1925.
48. Lal, A. Interaction of soil microorganisms with *Ophiobolus graminis* Sacc., the fungus causing the take-all disease of wheat. Ann. App. Biol. **26**: 247–261. 1939.
49. Loehwing, W. F. Root interaction of plants. Bot. Rev. **3**: 195–239. 1937.
50. Machacek, J. E., and Greaney, F. J. The effect of mechanical seed injury on the development of footrot in cereals. Canad. Jour. Res. **8**: 276–281. 1933.
51. ————, and ————. The "black-point" or "kernel smudge" disease of cereals. Canad. Jour. Res. **16**: 84–113. 1938.
52. McKinney, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Jour. Agr. Res. **26**: 195–218. 1923.

53. ————. Footrot diseases of wheat in America. U. S. Dept. Agr. Bull. 1347. 1925.
54. MEAD, H. W. Seed injury in wheat and infection by *Helminthosporium sativum* P. K. & B. Proc. World's Grain Exhib. & Conf., Regina, Canada 2: 278–282. 1933.
55. MEYER-BAHLBURG AND BINDFEIL. Betriebswirtschaftliche Hintergründe der Weizenfusskrankheiten. Deut. Landw. Pr. 62: 184. 1935. [Abstract in Rev. App. Myc. 14: 570. 1935 (Original not seen)].
56. MÜLLER-KÖGLER, E. Untersuchungen über die Schwarzbeinigkeit des Getreides und den Wirtspflanzenkreis ihres Erregers (*Ophiobolus graminis* Sacc.). Arb. Biol. Anst. (Reichsanst.) Berl. 22: 271–319. 1938.
57. OORT, A. J. P. De oogvlekkenziekte van de granen, Veroorzaakt door *Cercosporella herpotrichoides* Fron. Tijdschr. PlZiekt 42: 179–210. 1936. (English summary.) [Also abstract in Rev. App. Myc. 16: 29. 1937.]
58. PADWICK, G. W. Influence of wild and cultivated plants on the multiplication, survival and spread of cereal foot-rotting fungi in the soil. Canad. Jour. Res. 12: 575–589. 1935.
59. ————. Biologic strains of *Ophiobolus graminis* Sacc. Ann. App. Biol. 23: 45–56. 1936.
60. PADWICK, G. W. A growth factor influencing the development of *Ophiobolus graminis* Sacc. Sci. Agr. 16: 365–372. 1936.
61. ————. Note on the limitations of infection of wheat by ascospores of *Ophiobolus graminis* Sacc. A possible explanation. Ann. App. Biol. 26: 823–825. 1939.
62. RAINIO, A. J. Kauralaatujen punahome-*Fusarium roseum* Link. *Gibberella saubinettii* (Mont.) Sacc. Kestavyydesta. Valt. Maatalausk. Julk. 92: 24 pp. 1937. (German summary.) [Abstract in Rev. App. Myc. 16: 738. 1937.]
63. REMSBERG, RUTH E. The snowmolds of grain and grasses caused by *Typhula itoana* and *Typhula idahoensis*. Phytopath. 30: 178–180. 1940.
64. ROBERTSON, H. T. Maturation of foot and root tissue in wheat plants in relation to penetration by *Ophiobolus graminis* Sacc. Sci. Agr. 12: 575–592. 1932.
65. RUSSELL, R. C. Take-all studies. [In Rep. Dom. Bot. 1927: 105–108. Dept. Agr. Dom. Canada, Ottawa. 1928.]
66. ————. Studies of take-all and its causal organism *Ophiobolus graminis* Sacc. Dept. Agr. Dom. Canada, Ottawa. Bull. 170. 1934.
67. ————. Pathogenicity tests with cultures of *Ophiobolus graminis* Sacc. Sci. Agr. 19: 662–669. 1939.
68. ————, AND LEDINGHAM, R. J. Wheat seed testing from the pathological standpoint with special reference to embryo exposure. [To appear in Sci. Agr. at an early date.]
69. SALLANS, B. J. Methods of inoculation of wheat with *Helminthosporium sativum* P. K. & B. Sci. Agr. 13: 515–527. 1933.
70. ————. The use of water by wheat plants when inoculated with *Helminthosporium sativum*. Canad. Jour. Res. 18: 178–198. 1940.
71. ————. The relationship of weeds to losses caused by common rootrot in wheat. Sci. Agr. 20: 632–637. 1940.
72. SAMUEL, G. Whiteheads or take-all in wheat. Jour. Min. Agr. 44: 231–241. 1937.
73. SANFORD, G. B. *Colletotrichum graminicolum* (Ces.) Wils. as a parasite of the stem and root tissues of *Avena sativa*. Sci. Agr. 15: 370–376. 1935.
74. ————. Research on certain soil-borne diseases as affected by other microorganisms. Sci. Agr. 19: 609–615. 1939.
75. ————, AND BROADFOOT, W. C. Studies of the effects of other soil-

inhabiting microorganisms on the virulence of *Ophiobolus graminis* Sacc. Sci. Agr. 11: 512–528. 1931.
76. ———, AND ———. On the prevalence of pathogenic forms of *Helminthosporium sativum* and *Fusarium culmorum* in the soil of wheat fields and its relation to the rootrot problem. Canad. Jour. Res. 10: 264–274. 1934.
77. ———, AND CORMACK, M. W. Variability in association effects of other soil fungi on the virulence of *Helminthosporium sativum* on wheat seedlings. Canad. Jour. Res. 18: 562–565. 1940.
78. SCHAFFNIT, E. Beiträge zur Kenntnis der Fusskrankheiten des Getreides. I. Mitteilung. *Cercosporella herpotrichoides* Fron. als Ursache der Halmbruchkrankheit des Getreides. Phytopath. Zeit. 5: 493–503. 1933.
79. SIMMONDS, P. M. Seedling blight and footrot of oats. Dept. Agr. Dom. Canada, Ottawa. Bull. 105. 1928.
80. ———. A review of the investigations conducted in western Canada on rootrots of cereals. Sci. Agr. 19: 565–582. 1939.
81. ———. Root development in relation to rootrots in cereals. Sci. Agr. 19: 475–480. 1939.
82. ———, AND MEAD, H. W. The examination of wheat seed to determine the disease factor. Sci. Agr. 16: 175–179. 1935.
83. ———, RUSSELL, R. C., AND SALLANS, B. J. A comparison of different types of rootrot of wheat by means of root excavation studies. Sci. Agr. 15: 680–700. 1935.
84. SOLKINA, A. F. A study of the cycle of development of the fungus *Sclerotinia graminearum* Elen. Pl. Prot., Leningr. 18: 100–108. 1939. (In Russian with an English summary.) [Abstract in Rev. App. Myc. 18: 582. 1939.]
85. SPRAGUE, R. *Cercosporella herpotrichoides* Fron., the cause of the Columbia Basin footrot of winter wheat. Sci. 74: 51–52. 1931.
86. ———. The relative importance of *Cercosporella herpotrichoides* and *Leptosphaeria herpotrichoides* as parasites of winter cereals. Phytopath. 24: 167–168. 1934.
87. SPRAGUE, R. Relative susceptibility of certain species of Gramineae to *Cercosporella herpotrichoides*. Jour. Agr. Res. 53: 659–670. 1936.
88. ———. Influence of climatological factors in the development of *cercosporella* footrot of winter wheat. U. S. Dept. Agr. Cir. 451. 1937.
89. ———, AND FELLOWS, H. *Cercosporella* footrot of winter cereals. U. S. Dept. Agr. Tech. Bull. 428. 1934.
90. STEVENS, F. L. Footrot disease of wheat. Historical and bibliographic. Illinois Nat. Hist. Survey Bull. 13, 261–286. 1919.
91. STEYN, D. G. The toxicology of plants in South Africa. 1934.
92. TULLIS, E. C. *Leptosphaeria salvinii*, the ascigerous stage of *Helminthosporium sigmoideum* and *Sclerotium oryzae*. Jour. Agr. Res. 47: 675–687. 1933.
93. TUPENEVITCH, S. M., AND SHIRKO, V. N. Measures for preventing losses of winter cereals in the spring from *Sclerotinia graminearum* Elen. Pl. Prot. Leningr. 18: 85–99. 1939. (In Russian with an English summary.) [Abstract in Rev. App. Myc. 18: 581. 1939.]
94. VAN LUIJK, A. Untersuchungen über Krankheiten der Gräser. Meded. Phytopath. Lab. "Willie Commelin Scholten," Baarn. 13: 1–22. 1934. [Abstract in Rev. App. Myc. 14: 240. 1935.] (Original not seen.)
95. VANTERPOOL, T. C. Some species of *Pythium* parasitic on wheat in Canada and England. Ann. App. Biol. 25: 528–543. 1938.
96. ———. Present knowledge of browning rootrot of wheat with special reference to its control. Sci. Agr. 20: 735–740. 1940.
97. ———, AND SIMMONDS, P. M. The relation of browning rootrot to stem rust in causing injuries to wheat. Sci. Agr. 19: 81–82. 1938.

98. ———, and Truscott, J. H. L. Studies on browning rootrot of cereals. II. Some parasitic species of *Pythium* and their relation to the disease. Canad. Jour. Res. **6**: 68–93. 1932.
99. Volk, A. Untersuchungen über *Typhula graminium* Karst. Zeit. Pflkrankh. **47**: 339–365. 1937.
100. Weaver, J. E. Root development of field crops. 1926.
101. ———, Jean, F. C., and Crist, J. W. Development and activities of roots of crop plants. Carnegie Inst. Wash. Publ. 316. 1922.
102. Winter, G. Der Einfluss der physikalischen Bodenstruktur auf den Infektionsverlauf bei der Ophiobolose des Weizens. Zeit. Pflkrankh. **49**: 513–559. 1939.
103. Wollenweber, H. W., and Reinking, O. A. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekampfung. 1935.
104. Young, P. A. Sclerotium blight of wheat. Phytopath. **27**: 1113–1118. 1937.

NOTE ON THE USE OF THE TERM "PLASMO-DESMATA"

Publication of Dr. Meeuse's article in the May issue resulted in the editor's attention being called to the following note which Dr. J. H. Barnhart, Bibliographer of the New York Botanical Garden, had formulated just two years ago in reply to an enquiry. Its pertinency to the subject of Dr. Meeuse's article makes it desirable to call it to the attention of the readers of the BOTANICAL REVIEW:

PLASMODESMI

It is claimed that these threads were first clearly described and figured in 1880 by Eduard Tangl (Jahrb. Wiss. Bot. **12**: 170–190), but he coined no new word for them; in the title of his paper he called them "Communicationen," in his text, "Protoplasmafortsätze."

In 1882, Strasburger (Bau Zellhäute 246) called these structures "Plasmafäden," translated by Goodale, by Hillhouse, and by Coulter as "plasma threads," and this expression continued in general use for nearly twenty years.

Then, in 1901, Strasburger (Jahrb. Wiss. Bot. **36**: 503) proposed the word "Plasmodesmen." He observed that this word was better adapted than its predecessors to international use, but did not attempt to give its correct termination in any language other than German, and evidently assumed that no explanation of its derivation was needed. It was merely a substitute for "Plasmafäden," avoiding the Greek-German hybrid form of that word. The manifest derivation of the word is from "πλασμα" and "δεσμος", joined by the usual connecting vowel "–o–" so as to make "πλασμοδεσμος." This becomes in Latin "plasmodesmus" (plural "plasmodesmi"), and that is the correct English usage according to all English dictionaries recent enough to include the word. It might reasonably be anglicized to "plasmodesm" (plural "plasmodesms"), but this seems never to have been done, presumably because of the difficulty of clear enunciation of *desms*.

THE BOTANICAL REVIEW

VOL. VII JULY, 1941 No. 7

THE DIPLOID CELL AND THE DIPLOIDISATION PROCESS IN PLANTS AND ANIMALS, WITH SPECIAL REFERENCE TO THE HIGHER FUNGI

A. H. REGINALD BULLER
Professor Emeritus, University of Manitoba

CONTENTS

PART I

I. INTRODUCTORY REMARKS

Shortly after the middle of the nineteenth century, Hofmeister and others recognized that in the life-history of Mosses, Ferns, and

Flowering Plants a gametophytic generation and a sporophytic generation alternate with one another.

During the last quarter of the nineteenth century and the first few years of the twentieth century, cytological investigations on Mosses, Ferns, and Flowering Plants, made by Strasburger and others, revealed: that the *nuclei* of the cells of a gametophyte, like the nuclei of the gametes of animals, have a single set of chromosomes and are therefore *haploid;* and that the nuclei of the cells of a sporophyte, like the nuclei in the cells of the body of an animal, have a double set of chromosomes and are therefore *diploid.*

The words "haploid" and "diploid" were introduced by Strasburger in 1905 in the course of a discussion on nuclear division. Said he: "Finally it would perhaps be desirable if alongside the terms gametophyte and sporophyte, which can be used only for plants with the single and double chromosome number, there were set such other terms as would be suitable also for the animal kingdom. For this purpose I therefore permit myself to propose the words haploid and diploid, respectively haploidic and diploidic generation (bezw. haploidische und diploidische generation)." In 1907, Strasburger explained that the "id" in "haploid" and "diploid" served to connect the new terms with the "idioplasm" of Naegeli and the "id" and "idant" of Weismann.

The terms haploid and diploid have been universally accepted and they have been applied not only to generations, but also to nuclei, cells, tissues, and whole organisms.

After the chief cytological facts in the life-histories of Mosses, Ferns, and Flowering Plants had been brought to light, it was recognized that the *cells* of plants, just like those of animals, are divisible into two categories, *haploid* and *diploid.*

A *haploid cell* was regarded as being a *cell that contains a haploid nucleus.*

A *diploid cell* was regarded as being a *cell that contains a diploid nucleus.*

It was recognized that, in Mosses, Ferns, and Flowering Plants, the first diploid cell of a sporophyte is formed in the same way as the first diploid cell of the body of an animal, *i.e.* as a result of a haploid male nucleus entering an ovum and fusing with the haploid female nucleus.

II. SEXUALITY IN THE HIGHER FUNGI

In the closing decade of the nineteenth century and the first three decades of the present century it was discovered that, in the Higher Fungi (*Basidiomycetes,* including the Hymenomycetes, Gastromycetes, Uredinales, and Ustilaginales; *Ascomycetes,* including the Pyrenomycetes and Discomycetes), the nuclear phenomena accompanying the change from the haploid to the diploid state are more complicated and prolonged than those that had been observed by botanists in Mosses, Ferns, and Flowering Plants and by zoologists in animals.

In the Hymenomycetes (Mushrooms and Toadstools), Gastromycetes (Puff-balls, etc.), Uredinales (Rust Fungi), Ustilaginales (Smut Fungi), Pyrenomycetes, and Discomycetes, when two nuclei of opposite sex have just come into association with one another in the same cell, they do not fuse forthwith, but they divide simultaneously, thus giving rise to two pairs of nuclei, each of which resembles the parent pair. By repeated nuclear division of this kind, accompanied by hyphal growth and the formation of septa, a *series of cells* are formed all of which are *binucleate.* In the Ascomycetes, the binucleate cells make up the *ascogenous hyphae;* while, in the Basidiomycetes, the binucleate cells are the units of vegetative mycelia and the tissues of fruit-bodies. The union of two nuclei of opposite sex is long delayed, but eventually takes place: in the Ascomycetes in the young asci, and in the Basidiomycetes in the young basidia (Hymenomycetes, Gastromycetes) or probasidia (teleutospores of Uredinales, chlamydospores of Ustilaginales).

Coprinus lagopus is a small agaricaceous fungus that grows on horse-dung and passes through its life-history from spore to spore in about ten days. It is a good representative of the Hymenomycetes. *Coprinus lagopus* of Buller and his pupils is identical with the *C. fimetarius* of Bensaude (Buller, 1931).

As a result of cytological and experimental investigations made by Bensaude (1918), Buller (1930, 1931), Chow (1934), and others it was found for *Coprinus lagopus* that, after two multicellular haploid mycelia of opposite sex have been mated: (1) the mycelia unite with one another by means of hyphal fusions; (2) one or more nuclei pass from each mycelium into the other mycelium; (3) the invading nuclei move through the invaded mycelia,

dividing as they go, and passing from cell to cell probably *via* the central pores of the septa; and, finally, (4) the invading nuclei provide mates for all the nuclei of all the cells of the growing hyphae of both of the invaded mycelia. In this way what Bensaude called two *primary mycelia,* whose cells at first contain but a single haploid nucleus, come to be converted into what Bensaude called two *secondary mycelia* whose cells contain two haploid nuclei, one derived from one parent and the other from the other parent. The two nuclei in each cell are known as *conjugate nuclei* (Poirault and Raciborski, 1895).

When a cell of *Coprinus lagopus* or other Hymenomycete containing conjugate nuclei elongates apically or sends out a lateral branch and is about to divide into two cells, the pair of conjugate nuclei undergoes *conjugate nuclear division,* so that two daughter pairs of conjugate nuclei are formed. One of the daughter pairs becomes enclosed in one cell and the other daughter pair in the other cell. By long-continued growth in this way, the mycelium may develop into a plant body composed of thousands, hundreds of thousands, or even millions of cells all containing a pair of conjugate nuclei, $(n) + (n)$. And such a mycelium may live and spread in a tree-trunk (as in such a wood-destroying fungus as *Polyporus squamosus*), in the ground (as in *Marasmius oreades* and other Fairy-Ring Fungi), or in some other substratum for many years and fructify perennially.

The fructifications (fruit-bodies, sporophores) of the Hymenomycetes, commonly known as Agarics, Mushrooms, Toadstools, Boleti, Polypori (on the trunks of trees), etc., are made up of cells that, typically, resemble the cells of the mycelia on which they are borne, *i.e.* each of them contains a pair of conjugate nuclei.

The final activity of a fructification of a Hymenomycete is the production and liberation of millions of basidiospores (Buller, 1909, 1922, 1924). These are formed by the basidia and, typically, each basidium develops four sterigmata and four basidiospores.

Even the young basidia each contain a pair of conjugate nuclei; but in these cells the two nuclei soon fuse together, thus forming a diploid nucleus. This diploid nucleus, immediately after its formation, undergoes meiosis and so gives rise to four haploid nuclei. These make their way through the narrow necks of the sterigmata into the four basidiospores, and thus the basidiospores come to be haploid cells.

All the important phases of meiosis in Higher Plants (leptotene, parasynaptic conjugation, synaptic contraction, pachytene, diakinesis, etc.) have been observed in the Agaricaceae, *e.g. Pleurotus ostreatus* and *Cortinarius cinnamomeus* (Wakayama, 1930). Wakayama has shown that the first mitosis in the basidium is heterotypic and the second homotypic.

The conjugate nuclei in the mycelium and fruit-bodies of Hymenomycetes and Uredinales and in the ascogenous hyphae of Pyrenomycetes and Discomycetes are remarkable in that the two nuclei of each pair attract one another, keep near to one another, move about together in elongating hyphae, divide simultaneously, and yet are inhibited from fusing. Only in the young basidia and the young asci is this inhibition removed.

An ordinary diploid nucleus in a sporophyte of a Moss, Fern, or Flowering Plant or in an animal body contains two *genoms* or haploid sets of chromosomes, one derived from one parent and the other from the other parent; whereas a pair of conjugate nuclei of a mycelium or fruit-body of one of the Higher Fungi has the two genoms separate from one another, one set of chromosomes being enclosed in one nucleus and the other set in the other nucleus. A diploid nucleus of a Higher Plant or animal and a pair of conjugate nuclei of a Higher Fungus resemble one another in that their two genoms *divide simultaneously*. How the nuclei of a conjugate pair signal to one another across the cytoplasm that lies between them and arrange to divide at precisely the same time is a problem in cell physiology that at present remains unsolved.

While it is the rule that in the dikaryotic mycelium of Hymenomycetes, Uredinales, and other Basidiomycetes the two nuclei of each conjugate pair remain together and divide together, a few cases are known in which under certain conditions the two nuclei of a conjugate pair separate from one another and become enclosed in different cells, so that a dikaryotic mycelium comes to produce haploid branches. Here we have the phenomenon of de-diploidisation which will be discussed in a later Section.

In the dikaryotic mycelium of *Coprinus lagopus* and very many other Hymenomycetes, the division of a pair of conjugate nuclei is associated with cell-division and the formation of *two septa*. One septum is formed across the middle of the original apical hyphal cell and the other across a short semicircular hypha that develops dur-

ing cell-division in such a way as to connect the two cells together. This short semicircular hypha is known as a *clamp-connexion.* Functionally, however, it is what we may call a *by-pass hypha* (Bessey, 1935). Each of the septa, as in the septa of the Higher Fungi in general, has a minute (about 1 μ wide) open pore in its centre, and through the two pores of the two septa cytoplasm flows when on its way to fill up the cavities of new hyphae being added to the vegetative mycelium or being formed in developing fruit-bodies. The two septa between two successive cells may be regarded collectively as comparable with a sieve-plate separating and yet connecting two cells of a sieve-tube; but, whereas a sieve-plate in one of the Higher Plants has many pores, the two septa under discussion collectively have but two pores.

Greis (1937) concluded that the clamp-connexions of the Hymenomycetes and other Basidiomycetes are functionless structures; but, when formulating his arguments, he was unaware that the two septa of a clamp-connexion both have a central open pore through which labile cytoplasm can flow.

Clamp-connexions were discovered and called *Schnallenzellen* by Hoffmann in 1856. That they are present in the secondary mycelia of very many Hymenomycetes and Gastromycetes was shown by the observations of Bail (1856), de Bary (1859, 1866, 1884), Tulasne (1861), Robert Hartig (1874, 1878, 1885), Brefeld (1877, 1888, 1889), and others; but it is to the work of Kniep (1915) and Bensaude (1918) that we are indebted for the important fact that conjugate nuclear division takes place whenever a clamp-connexion is formed. The pores in the septa of a clamp-connexion were first observed by Wahrlich (1893). The flow of cytoplasm through the pores of septa of the Higher Fungi, including the Hymenomycetes, was treated of in detail by myself in 1933; and, in the same year, I described the successive stages in the formation of a clamp-connexion on a *living* hypha of *Coprinus lagopus* and *C. sterquilinus* as seen under the microscope and showed that the last stage in the process is a *hook-to-peg* hyphal fusion.

Brefeld (1877) observed that the hook of a clamp-connexion arises on the side of a terminal hyphal cell and grows *backwards* away from the apex of the cell, and not more or less forwards as does an ordinary hyphal branch. This curious mode of development secures that the terminal elongating cell shall always contain

two nuclei and thus have its nucleo-plasma ratio kept fairly constant (Buller, 1933). If the hook grew forward instead of backwards, the terminal cell, which ordinarily is binucleate, would (as I showed by means of a diagram) become temporarily uninucleate whenever, during the formation of a clamp-connexion, a nucleus is imprisoned in the hook-cell; and the terminal cell would remain uninucleate until the hook-cell had fused with it and the hook-cell nucleus had thus become free to join its conjugate mate.

To explain the backward growth of the hook no theory alternative to my own has so far been proposed. To my theory Noble (1937) has raised certain objections. These seem to be of little weight, but here no reply can be made to them.

Owing to the fact that the hook of a clamp-connexion grows backwards instead of forwards, the growing-point of a dikaryotic hypha has behind it always a full set of pairs of homologous chromosomes instead of sometimes a full set and at other times a half set. Since, as will be shown later, both of the genoms of a conjugate pair of nuclei affect the growth and development of the cell in which they lie, the constant presence of a pair of conjugate nuclei in the apical cell of a hypha may well be of considerable importance.

It is generally agreed that, normally, in heterothallic Hymenomycetes, clamp-connexions are not formed on haploid mycelia.

From what has just been said it is clear that, in Hymenomycetes like *Coprinus lagopus*, a clamp-connexion is the outward and visible sign of the presence of conjugate nuclei in each of the cells between which the clamp-connexion lies. This fact is of great importance in making genetic experiments, for it enables the worker, without going to the trouble of fixing and staining his material, to determine very quickly by microscopic inspection whether or not any given mycelium has passed from the primary (haploid) to the secondary (diploid) condition.

In many Hymenomycetes, *e.g. Merulius lacrymans* (Hartig, 1885; Falck, 1912), *Corticium varians, Hypholoma fasciculare, Polystictus versicolor,* and *Clavaria pistillaris* (Kniep, 1915) and in the Gasteromycete *Sphaerobolus stellatus* (Buller, 1934), clamp-connexions are present in the secondary mycelium and in the fruit-bodies up to the bases of the basidia. In other Hymenomycetes, *e.g. Coprinus lagopus* (Buller, 1924, 1931), clamp-connexions are present in the secondary mycelium but not in the fruit-bodies;

while, in still other Hymenomycetes, *e.g. Coprinus curtus* (Mounce, 1921) and *Psalliota campestris* (cultivated form), clamp-connexions are found neither in the secondary mycelium nor in the fruit-bodies (Hirmer, 1920; Buller, 1922). In the gelatinous Tremellaceae, Auriculariaceae, and Dacryomycetaceae clamp-connexions may not often be present. Brefeld (1888, 1889) did not observe them in these groups, and I failed to find them in *Dacryomyces deliquescens* (1922); but Kniep (1928) saw them in *Sebacina calcea* (Tremellaceae) and *Auricularia mesenterica*. Numerous investigations made by various workers all go to show that, in the Uredinales, clamp-connexions are completely absent. These structures were also not seen by Couch (1938) in the Septobasidiales. Thus while clamp-connexion formation is always associated with conjugate nuclear division, the converse is not true; for conjugate nuclear division may take place without the formation of clamp-connexions.

It was claimed by Seyfert (1927) that clamp-connexions occur in the secondary mycelium of various Ustilaginales, including *Ustilago Vuijckii, U. zeae* (his *U. maydis*), *U. longissima, U. grandis, U. pustulata, U. striaeformis, Doassansia sagittariae, Tilletia Rauenhoffii, Urocystis anemones, Entoloma calendulae, E. ranunculi, E. chrysosplenii, Tubercinia trientalis,* and *T. primulicola.* Stakman and Christensen (1927) and also Hanna (1929) thought they had seen clamp-connexions in *Ustilago zeae;* but it is now clear from the work of Christensen (1931) and especially Sleumer (1932), that in *U. zeae* there are no true clamp-connexions whatsoever. Thus Seyfert's findings with *U. zeae* have not been confirmed. On this account one cannot accept his conclusion that clamp-connexions are present in other Ustilaginales. To what extent, if any, clamp-connexions occur in this group can be decided only by further and more critical observations. The investigator has to be on his guard not to mistake "clamp-like structures" (Christensen, 1931) for true clamp-connexions.

False or incomplete clamp-connexions (Brunswik's *Pseudoschnallen*) in which the hook does not become fused with the parent hypha are produced in some Hymenomycetes, *e.g. Coprinus picaceus,* in certain so-called illegitimate unions (Brunswik's *Durchbrechungskopulationen*), such as $(AB) \times (Ab)$ and $(ab) \times (Ab)$, where two haplonts in a combination have a factor in common

(Brunswik, 1924; Oort, 1930; Vandendries, 1930, b; Quintanilha, 1933; Biggs, 1938). Illustrations of false clamp-connexions have been given by Brunswik for *Coprinus lagopus* (his *C. fimetarius*), by Vandendries for *Psathyrella disseminata,* and by Biggs for *Peniophora ludoviciana.*

In some Hymenomycetes whose secondary mycelium bears clamp-connexions, *e.g. Stereum hirsutum* (Kniep, 1928) and *Coniophora cerebella,* the hyphal cells contain more than one pair of conjugate nuclei and, when cell-division takes place, two, three, or more clamp-connexions may be formed simultaneously between the daughter cells. In *C. cerebella,* in very thin hyphae there are single clamp-connexions; but, as thicker and thicker hyphae are developed, the number of clamp-connexions in a single whorl rises from two to as many as eight (Falck, 1911).

Hirmer (1920) made observations on the cytology of the Common Mushroom, *Psalliota campestris,* as follows. The cells of the stipe and pileus-flesh contain not a pair of conjugate nuclei, but groups of individual nuclei numbering from two up to eleven in each group. These groups of nuclei divide conjugately so that a group of say six nuclei, on division, would produce two daughter groups of six nuclei. There is a gradual reduction in the number of nuclei in the groups in the cells of the lamellae (gills) until at last the normal number two (a single pair of conjugate nuclei) is attained, and this is accomplished before the subhymenium is formed. This allows of a single pair of conjugate nuclei entering each basidium. Here nuclear fusion takes place in the usual manner.

The sexual reactions of certain Hymenomycetes, *e.g. Coprinus radians* (Vandendries, 1924) and *C. Rostrupianus* (D. Newton, 1926, b), are governed by *one pair* of factors (*Aa*), and the four spores on each basidium are (*A*), (*A*), (*a*), (*a*). Such a species has been called bisexual or *bipolar.* Like cannot interact sexually with like, so that the only successful matings are (*A*) × (*a*).

In other Hymenomycetes, *e.g. Schizophyllum commune* (Kniep, 1920, 1922, 1923) and *Coprinus lagopus* (Hanna, 1925), the sexual reactions are governed by two pairs of factors (*Aa*) and (*Bb*), and the four spores on a basidium, as was shown by D. Newton (1926, a) for *C. lagopus,* may be (*AB*), (*AB*), (*ab*), (*ab*), or (*Ab*), (*Ab*), (*aB*), (*aB*), or (*AB*), (*ab*), (*Ab*), (*aB*). Thus, on the

hymenium, there are three kinds of basidia. Such a species has been called quadrisexual or *tetrapolar*. Like factors repel one another, so that the only successful sexual matings are $(AB) \times (ab)$ and $(Ab) \times (aB)$. One kind of haplont can interact sexually with only one of the other three kinds of haplonts. The fusion nucleus in every basidium contains all the four sex factors and is therefore $(AaBb)$.

One may ask: how comes it in *Coprinus lagopus* that, while some basidia bear only two kinds of spores, (AB), (AB), (ab), (ab) or (Ab), (Ab), (aB), (aB), other basidia bear all the four kinds of spores (AB), (ab), (Ab), (aB)? My pupil, D. Newton (1926, b), suggested that: in basidia bearing only two kinds of spores, reduction of the two pairs of chromosomes takes place at the first nuclear division; whereas, in basidia bearing four kinds of spores, there is a reduction of one pair of chromosomes at the first nuclear division and a reduction of the other pair of chromosomes at the second nuclear division.

In the Ascomycetes, also, as a result of crossing races differing in two pairs of factors that can be inherited independently, in some asci there are two kinds of ascospores while in other asci there are four kinds. Here, again, the reduction problem presents itself. Lindegren (1933) was the first to suggest that, in *Neurospora crassa* and similar species, segregation at the second division of the fusion nucleus is due to *crossing-over*. By genetic experiments on *N. crassa*, in which he employed linked genes, he proved that second-division segregation percentages are accurate measures of crossing-over percentages and, on this basis, he (1936) published a six-point map of the sex chromosome. In 1939, the Lindegrens described another chromosome with four loci.

Dodge (1940) has recently suggested that Newton's findings with *Coprinus lagopus* can be explained on the same basis as those of Lindegren with *Neurospora crassa, i.e.* there is not a reduction of one pair of chromosomes at the first nuclear division and a reduction of the other pair of chromosomes at the second division; but, rather, "by means of a simple cross-over, segregation of factors can occur in the second division though the chromosomes disjoin in the first division." Dodge's explanation of the results of Newton and of similar results obtained by Brunswik (1926) seems a better one than that of myself and Newton, and I am therefore glad to accept it.

In *Coprinus lagopus* (Brunswik's and Oort's *C. fimetarius*) and some other species of Hymenomycetes, in illegitimate combinations where there is one factor in common, *e.g.* $(AB) \times (Ab)$ or $(aB) \times (ab)$, the two mycelia, especially when they are very young (Brunswik, 1924), may break through the usual rules and interact sexually with one another to a certain degree (Brunswik's *Durchbrechungskopulationen,* 1924). The result is that on the mycelium there are formed, not a clamp-connexion between every two cells as in a legitimate combination such as $(AB) \times (ab)$, but a certain number of clamp-connexions, some true and some false, in an irregular manner. To what extent the mycelium of such an illegitimate combination has the nuclei merely mixed or in conjugate pairs remains to be decided by cytological investigation. Heldmaier (1929), working with *Schizophyllum commune* and *Collybia velutipes,* has shown that the number of clamp-connexions in some illegitimate combinations may be increased by the action of poisonous substances, high temperature, and low temperature, but not by X-rays. Oort (1930) found that the fruit-bodies of *Coprinus lagopus* produced on a mycelium of an illegitimate combination, say $(AB) \times (Ab)$, are always *haploid* and also that they yield two kinds of spores only, here (AB) and (Ab), resembling the two kinds represented in the two spores from whose two mycelia the fruit-bodies were derived. Oort also found that some of the basidia of one of the haploid fruit-bodies produce four spores of one sexual kind, while other basidia produce four spores of the other sexual kind: in our example some basidia produce four (AB) spores while other basidia produce four (Ab) spores. Oort concluded that the haploid fruit-bodies developed by such an illegitimate combination as $(AB) \times (Ab)$, since they bear haploid basidia of two different kinds, are to be regarded as chimaeras.

The Higher Fungi, typically, are *heterothallic* (haplodioecious, Hartmann, 1918, 1939), *i.e.,* they have the sexes separate from one another in different haploid mycelia. Among heterothallic species may be mentioned *Coprinus lagopus, Puccinia graminis, Ustilago zeae, Neurospora sitophila,* and *Ascobolus magnificus.* Except in Section III, it is with such species as these that we shall be concerned.

In both bipolar and tetrapolar Hymenomycetes there are so-called *geographical races* most of which are completely interfertile

with one another. Thus in *Coprinus lagopus* (Brunswik's *C. fimetarius*) Brunswik obtained twenty-seven such races, all of which were completely interfertile. These can be represented by the symbols for their four kinds of haplonts: Race I, (AB), (ab), (Ab), (aB); Race II, (A^1B^1), (a^1b^1), (A^1b^1), (a^1B^1); Race III, (A^2B^2), (a^2b^2), (A^2b^2), (a^2B^2); etc. Then a haplont of any one race, say (A^2B^2), is fertile with all four haplonts of Race I or of Race II or of any other geographical race. Geographical races will be further treated of in Section XXIII.

To explain how sex is determined in the Hymenomycetes in general there are two different theories, one proposed by Kniep and the other by Hartmann.

Kniep (1929, a, b) explains sexual bipolarity in a Hymenomycete by assuming that the factors A and a which determine sexual differences are represented by genes located in two homologous chromosomes and that, when reduction of the diploid nucleus (Aa) of a basidium takes place, the pair of allels splits apart so that the basidium produces two spores with the A factor and two with the a factor. Like haplomycelia do not interact sexually. Hence the only successful matings are $(A) \times (a)$.

Kniep explains tetrapolarity as a case of dihybridism, the sex factors being represented by genes located in two pairs of homologous chromosomes instead of one pair. Therefore, when the diploid nucleus $(AaBb)$ of a basidium undergoes reduction, each of the four haploid nuclei comes to contain one factor of each of the two pairs Aa and Bb. Thus the four spores on any basidium may be: (AB), (AB), (ab), (ab) or (Ab), (Ab), (aB), (aB) or (AB), (ab), (Ab), (aB). Haplomycelia with two factors or even one factor in common do not interact sexually. Hence the only successful matings are $(AB) \times (ab)$ and $(Ab) \times (aB)$. In both of these matings all four of the factors are brought together in the fusion nucleus $(AaBb)$ of the basidium.

Kniep explains multipolarity of geographical races within a species as being due to multiple allelomorphy. Thus, in a bipolar species, for each race, the same pair of homologous chromosomes is involved. If we represent the races as: (A), (a); (A^1), (a^1); (A^2), (a^2); (A^3), (a^3); etc., then (for example) A^1 will mate successfully with A, A^2, A^3, etc., as well as with a, a^2, a^3, etc., because, by mutation, A^1 has become quantitatively sufficiently different from A, A^2, A^3, etc., to permit of sexual union.

Hartmann (1929, 1939), accepting ideas of Correns based on investigations on sex in certain Phanerogams, has sought to elaborate a theory of sex determination that would be applicable to plants and animals in general from the lowest to the highest. He holds that, in every gamete or haploid equivalent, there is present the potencies of both the male and the female sexes, represented by **AG** (**A** for male potency, **G** for female potency) and that the male or female potency actually exhibited by any gamete is determined in haplodioecious organisms, *e.g. Coprinus lagopus,* or in diplodioecious organisms, by heritable genetic factors that he calls *realizators.* In haplomonoecious (sexes mixed in the haplophase) and diplomonoecious (sexes mixed in the diplophase) organisms, sex determination is due not to heritable genetic factors, but to other inner or outer conditions.

Hartmann explains bipolarity in a Hymenomycete as follows. The diploid nucleus in a basidium contains the bisexual potency **AG** and, located on a pair of homologous chromosomes, the realizators M and F. When reduction takes place, each of the four haploid nuclei contains **AG** and either M or F. The male realizator M suppresses the **G** potency and thus causes a spore containing M to be male, **A**; while the female realizator F suppresses the **A** potency and thus causes a spore containing F to be female, **G**. Thus Kniep's (A) and (a) haplonts, on Hartmann's theory, are respectively male and female haplonts.

As Kniep (1929, b) has pointed out, difficulties for Hartmann's theory arise as soon as one attempts to explain the interfertility of geographical races. Hartmann supposes that the M and F realizators of one race differ from the realizators of other races, so that in differing races the realizators M and F, M^1 and F^1, M^2 and F^2, M^3 and F^3, etc., suppress maleness or femaleness in differing degrees. If Kniep's haplonts (A) and (a) of one race are respectively male and female, then also the haplonts (A^1) and (a^1), (A^2) and (a^2), (A^3) and (a^3), etc., of other races are respectively male and female. Since (for example) the haplont (A) mates successfully with (A^1) or (A^2) or (A^3), etc., we have a male haplont that interacts successfully with a series of other male haplonts differing from it in degrees of male sexuality, and so contributes to the production of many races each of which has a male tendency only and a male tendency differing from that of other races. As one takes

into consideration each additional geographical race, the explanation of sex determination, on Hartmann's theory, becomes more and more involved in relative sexuality and correspondingly more difficult and less probably correct.

Further complications for Hartmann's theory arise as soon as one attempts to apply it to a tetrapolar species, *e.g. Coprinus lagopus;* for such a species must be interpreted in terms of bisexuality, with two of the four kinds of haplonts relatively male but differing in their degrees of maleness and the other two kinds of haplonts relatively female but differing in their degrees of femaleness (*cf.* diagrams used by Hartmann, Vandendries, Kniep, and Quintanilha). In the tetrapolar species, *Coprinus lagopus,* twenty-seven completely interfertile geographical races are known. To explain how all these races are related to one another from the point of view of sex determination seems, on Kniep's quantitative theory, comparatively simple but, on Hartmann's qualitative theory, *i.e.* on a basis of relative maleness and femaleness, very difficult.

To explain sex determination in the Hymenomycetes, Vandendries (1930, a, b) sought to use Hartmann's theory, but encountered difficulties. On the other hand, for the reasons given above and for other reasons that can not be dealt with here, Kniep (1929, b) and Quintanilha (1935) have not been able to accept Hartmann's theory in so far as it concerns the Hymenomycetes.

III. HOMOTHALLISM

Relatively few of the Hymenomycetes are homothallic, *i.e.,* have the sexes combined in a single individual, so that each individual is self-fertile. This is indicated by the lists of homothallic and heterothallic Agaricaceae as given by Kniep (1928) and by the more recent investigations of various workers. As a result of her cultural studies of the Thelephoraceae and other related lower Hymenomycetes, Biggs (1938) has listed 29 species belonging to Corticium, Cytidia, Odontia, Peniophora, Radulum, and Stereum in all of which the type of sexuality has been determined. Only one of these species (*Corticium coronilla:* group II) proved to be homothallic, and all the others were found to be heterothallic. Thus it would seem that, in the Thelephoraceae and its allies, just as in the Agaricaceae, the heterothallic species greatly outnumber the homothallic.

In the Ustilaginales heterothallism is the rule. In the Uredinales, in heterothallic genera such as Puccinia, some homothallic species are known. The indications are that the Pyrenomycetes and Discomycetes are prevailingly heterothallic.

Among the Discomycetes is the large, varied, and economically important genus Sclerotinia. Heterothallism in this genus was discovered by Drayton. Drayton (1932, 1934) obtained isolates of *Sclerotium Gladioli* and mated them in all possible ways by "spermatizing" the "receptive bodies" on the stroma of each isolate with microconidia derived from the other isolates. From certain combinations he obtained ascocarps of a Sclerotinia that he called *Sclerotinia Gladioli.* Other matings, involving the employment of mycelia derived from germinated ascospores, gave a similar result Thus it was clearly indicated that *S. Gladioli* is heterothallic, some thalli being (+) in sex and others (−).

Drayton (1937) also obtained isolates of *Botrytis convoluta* that causes a destructive disease of the Garden Iris and mated them by "cross-spermatizing" the receptive structures on the sclerotia of each isolate with microconidia derived from the other isolates. From certain combinations he obtained the perfect stage of *B. convoluta* in the form of ascocarps of a Sclerotinia that he called *Sclerotinia convoluta.* He then germinated the ascospores, obtained monosporous mycelia and, by cross-spermatization, obtained ascocarps from certain combinations. Thus he proved that the ascospores are haploid and unisexual.

Groves and Drayton (1939) as a result of "cross-spermatizing" the sclerotia of isolates of *Botrytis cinerea* have shown that the perfect stage of this fungus is a Sclerotinia (unnamed as yet) and, therefore, that this Sclerotinia is heterothallic.

On overwintered leaves of Narcissus "Soleil d'Or" that had been killed by *Botrytis polyblastis* Gregory (1938) obtained a Sclerotinia that he called *Sclerotinia polyblastis* and then found that single ascospores each gave rise to *Botrytis polyblastis* mycelia. Doubtless, therefore, *S. polyblastis,* like the Sclerotinia associated with *Botrytis cinerea,* is heterothallic.

Thus the investigations of Drayton, Groves and Drayton, and Gregory indicate that Sclerotinia as a genus is heterothallic rather than homothallic and that Botrytis mycelia are sclerotinial haplonts.

The sexual condition of the Saccharomycetales has been discussed by Guilliermond (1931, 1937; 1940, on p. 4 "heterothallic" should read "homothallic"). He concluded that: (1) yeasts such as *Schizosaccharomyces octosporus, Zygosaccharomyces Barkeri, Zygopickia Chevalieri,* and *Nadsonia fulvescens* in which conjugation of vegetative cells precedes the formation of the ascus are *homothallic* because differentiation of the sexes takes place in cells which have been derived from a single ascospore; and (2) yeasts such as *Saccharomycodes Ludwigii* in which the ascospores conjugate in pairs while still in the ascus are *heterothallic*.

The genetics and heterothallism of *Saccharomycodes Ludwigii* have been investigated by Winge and Laustsen and by Manuel. In *S. Ludwigii,* in each ascus, there are four ascospores in a row in coherent pairs. The spores of each pair act as gametes and fuse together. The two zygotes germinate and give rise to oval yeast-cells, each containing a diploid nucleus, that bud indefinitely. Winge and Laustsen (1939, a), with the help of a micromanipulator, isolated a number of ascospores, sowed them separately, and obtained monosporous cultures. They then found that each ascospore developed in one of four different ways: (1) with normal growth and short (oval) cells (*Nl*), (2) with normal growth and long cells (*NL*), (3) with very limited growth (a few cells only) and short cells (*nl*), or (4) with very limited growth and long cells (*nL*). From a study of the mode of development of all the four spores removed from single asci it became clear that: (1) the two nuclei of the two ascospores of each conjugating pair are in some asci (*NL*) and (*nl*) and in other asci (*Nl*) and (*nL*); (2) the nucleus of each of the two zygotes formed within an ascus, and also the diploid nucleus of every normal yeast-cell budded off from a zygote, is heterozygous (*NnLl*); and (3), in the normal diploid yeast-cells, the factor (*N*) for normal growth is dominant over the factor (*n*) for very limited growth, and the factor (*l*) for the production of short oval cells is dominant over the factor (*L*) for the production of long cells. Thus it is indicated that the ascospores that unite to form a zygote are genotypically always different from one another. We may therefore regard the conjugating ascospores as being of opposite sex, one (+) and the other (–). The yeast-cells in colonies that have been derived from single ascospores remain haploid, never become diploid, and are unable to produce asci

and ascospores. Manuel (1939) isolated the four spores from single asci and, on mating the ascospores of each pair, almost constantly obtained conjugation. On the other hand, on mating ascospores of different pairs, she obtained much less conjugation (Guilliermond, 1940, reported 50 per cent.). Thus the observations of Winge and Laustsen and of Manuel all point to the conclusion that *Saccharomycodes Ludwigii* is heterothallic and that sex in this species is determined, just as in the Pyrenomycete *Neurospora sitophila,* genotypically during meiosis in the young ascus.

In *Saccharomycodes Ludwigii,* as observed by Manuel (1939), the yeast-cells derived from isolated ascospores do not fuse with one another in any combination, so that in this species the ascospores alone act as gametes. Since the asci do not discharge their ascospores and the ascospores of isolated asci conjugate in pairs *in situ,* one might at first suppose that, under natural conditions, the crossing of different strains or races of *S. Ludwigii* could not take place. However, Guilliermond (1937, Fig. 12) observed that, when the asci are crowded together, ascospores of two different asci sometimes fuse together. Thus the crossing of different strains or races is possible. The distance across which isolated ascospores of opposite sex stimulate one another and grow across so as to effect conjugation was observed by Manuel (1939) to be 5–10 μ.

The genus Saccharomyces, which includes *S. cerevisiae* (bread yeasts) and *S. ellipsoideus* (wine yeasts), according to Guilliermond (1937) and Winge and Laustsen (1937), is homothallic. Hansen (1891) observed that in *S.* (*ellipsoideus* Hansen) *forma Johannisberg II, S. ellipsoideus* Hansen, and *S. validus* Hansen, the ascospores in the asci usually conjugate in twos. It was then supposed that single ascospores that have germinated without any conjugation can reproduce the species parthenogenetically. Recently, the fate of unpaired ascospores of Saccharomyces species has been elucidated by Kruis and Šatava (1918), Šatava (1918, 1934), Winge, and Winge and Laustsen. The original papers of Kruis and Šatava and of Šatava were published in Czechian journals and on this account, unfortunately, they were unknown to Kniep (1928) and they remained unknown to Guilliermond and Winge until Winge (1935) had completed his paper on the haplophase and diplophase in some Saccharomycetes. In a postscript to this paper, Winge cites the papers of the Czech authors.

Winge (1935) discovered independently, thereby confirming similar observations made by Kruis and Šatava (1918), that single ascospores of *Saccharomyces ellipsoideus* may germinate and produce "short-shoot," small, more rounded, haploid yeast-cells that conjugate with one another and so produce zygotes that immediately give rise to normal oval diploid yeast-cells each containing a diploid nucleus. Thus, in the life-history of *S. ellipsoideus*, starting from a single ascospore, a short haploid phase is followed by the formation of a few or of multiple zygotes and a subsequent long diploid phase (*cf.* Guilliermond's life-history diagrams, 1937). However, there is another possibility for the fate of unconjugated ascospores. Winge and Laustsen (1937) discovered that single haploid ascospores of *S. ellipsoideus,* instead of giving rise to haploid yeast-cells that conjugate, may become diploid when germinating owing to the fusion of the first daughter nucleus with the mother nucleus. A diploid nucleus is sent into the first bud and thus all the cells budded off from the originally haploid ascospore come to be normal oval yeast-cells each provided with a diploid nucleus. Since we now know that a single haploid ascospore of *S. ellipsoideus,* either by means of a union of two haploid nuclei in its interior or by producing haploid cells that subsequently conjugate, gives rise to diploid cells, we must regard *S. ellipsoideus* as being homothallic; and, since other Saccharomyces species have a life-history similar to that of *S. ellipsoideus* (Winge, 1935), we must regard Saccharomyces as being a homothallic genus.

Notwithstanding that *Saccharomyces ellipsoideus, S. cerevisiae,* etc., are homothallic, the possibility of crossing different strains or races is still present; for, when asci are crowded together, either (1) two ascospores belonging to different asci of two different races may conjugate or (2) two haploid yeast-cells produced by ascospores of two different races may conjugate. Winge and Laustsen, by mating ascospores, have actually produced a number of yeast hybrids (*vide infra,* Section VII). In *S. ellipsoideus* there is much variability. Thus Winge and Laustsen (1937) found that the four yeast types originating from the four spores of a single ascus may differ greatly in appearance in giant-colonies on gelatine. It thus appears that genetic segregations take place during the formation of ascospores in an ascus. Winge and Laustsen (1937) concluded that: "From observations on the appearance of

giant-colonies it is established that heterozygosity in more than one gene is decisive for the segregations, and that crossing-over may take place frequently during the reduction division in the ascus." Winge (1935) also concluded that, in *S. ellipsoideus,* although conjugation takes place between ascospores or yeast-cells, "there is no demonstrable difference of sex between the fusing cells, neither genotypical nor phenotypical."

In the Laboulbeniales, as Thaxter (1896–1931) found, there are a number of genera in which the species are dioecious: two coherent ascospores of which one is often larger than the other, after extrusion from an ascus and deposition on a host-insect, germinate side by side, and the smaller spore gives rise to a plant bearing "spermatia (antherozoids)" and the larger to a plant bearing ascogonia each provided with a trichogyne. Here we have heterothallism expressed as sexual dimorphism, and the determination of sex takes place genotypically during meiosis in the young ascus. Many other genera of the Laboulbeniales are differently organised, for they are monoecious (hermaphrodite): from single ascospores there are developed individual plants that bear both spermatia and ascogonia. It may be that a monoecious species is homothallic so that each individual fungus plant derived from a single ascospore is self-fertile. On the other hand, it may be that a monoecious species is heterothallic and thus resembles *Neurospora sitophila.* If so, some monosporous plants are (+) and others (–), each plant is self-sterile, and fertility results only from the transference of spermatia from one plant to a trichogyne of another plant. The sexual process, just as in the Sclerotiniae investigated by Drayton, would be accomplished only by "cross-spermatization". Whether the monoecious species of the Laboulbeniales are homothallic or heterothallic awaits decision by means of an experimental investigation. The cytology of the monoecious Laboulbeniales has been discussed by Dodge (1927).

Assuming that the monoecious species of the Laboulbeniales are heterothallic, then, as Dr. B. O. Dodge has suggested to me, a monoecious species might give rise to a dioecious species by means of a crossing-over during meiosis: the genes for spermatium-production and the genes for ascogonium-production, which we may assume to be ordinarily close together on the same chromosome, might become separated from one another and separated in such a

way that the spermatium-production gene would become linked with say the (–) sex and the ascogonium-production gene with the (+) sex. Thus, perhaps, in the Laboulbeniales, the dioecious species have been derived from the monoecious species.

Two kinds of homothallic species may be distinguished: (1) those that are *haplomonoecious* (Hartmann, 1918, 1939), and (2) those that are sexually heterokaryotic or *miktohaplontic* (Kniep, 1928, 1929).

(1) In haplomonoecious homothallic species, the life-history starts: with a single haploid ascospore, *e.g. Pyronema confluens* (Claussen, 1912); or with a haploid ascospore that may or may not fuse with another ascospore, *e.g. Saccharomyces ellipsoideus* (Winge, 1935; Winge and Laustsen, 1937); or with a single haploid basidiospore, *e.g. Coprinus sterquilinus* (Mounce, 1921, 1922) and *Puccinia malvacearum* (Ashworth, 1931; Brown, 1940).

(2) In sexually heterokaryotic or miktohaplontic homothallic species, the life-history starts: with a single bisexual ascospore containing from the first haploid nuclei of both sexes, *e.g. Neurospora tetrasperma* (Dodge, 1927), *Pleurage anserina* (Dowding, 1931, a), and *Gelasinospora tetrasperma* (Dowding, 1933); or with a single bisexual basidiospore containing from the first haploid nuclei of both sexes, *e.g.* the basidially bisporous forms of the otherwise basidially quadrisporous heterothallic Hymenomycetes *Coprinus ephemerus, Galera tenera,* and *Naucoria semiorbicularis* (Sass, 1929), the basidially bisporous Rust *Puccinia arenariae* (Lindfors, 1924), and the basidially bisporous (bisporidial) Smut *Cintractia montagnei* (Rawitscher, 1922).

In the homothallic species *Coprinus sterquilinus,* nuclear fusion in a basidium is followed by meiosis, the passage of a single haploid nucleus into each of the four young basidiospores, and the division of the nucleus of each basidiospore into two nuclei. Each basidiospore gives rise to a mycelium that is at first multinucleate and then dikaryotic and provided with clamp-connexions (Brunswig, 1924; Buller, 1924, 1931). Jackson (1935) holds that, in homothallic species like *C. sterquilinus,* the two nuclei of each conjugate pair, since they have been derived from the division of a single haploid nucleus, must chromosomally be exactly alike. However, we know that, normally, in heterothallic species like *C. lagopus,* two like nuclei in any single haplont do not come together to form a con-

jugate pair, and that a conjugate pair is formed only by two nuclei of opposite sex. One may therefore suppose that, even in *C. sterquilinus,* there must be some physiological difference between the two nuclei of each conjugate pair or they would not be associated with one another. That a single haploid nucleus can give rise to nuclei with differing sexual potentialities is shown by certain observations of Hanna (1925, 1928) which will now be cited. In *C. lagopus,* Hanna obtained a sexual mutation in a haplont derived from a single basidiospore of such a kind that the haplont developed clamp-connexions and thus appeared to be bisexual. Fruit-bodies developed on this mycelium. Ten monosporous mycelia derived from as many basidiospores taken from two of the fruit-bodies were investigated experimentally. "It was found that the mycelia were not all of one and the same sex, as was to be expected if the fruit-bodies had been haploid, but that they fell into four groups, thus proving that the fruit-body was diploid. Moreover, one of the groups of mycelia was completely fertile with the other three groups, thus indicating that a mutation had taken place with the production of a new sexual strain." In this case, therefore, the two nuclei of a conjugate pair in the original mutating haplont must have been of opposite sex, and not both of the same sex and chromosomally exactly similar to the haploid nucleus of the basidiospore from which the mutant mycelium was derived.

Sass (1929, a) has suggested that, in *Coprinus sterquilinus:* the four spores of a basidium each receive a diploid nucleus; nuclear reduction and the segregation of sex factors takes place in the spores; and thus the mycelium derived from each basidiospore receives both (+) and (−) nuclei. This hypothesis was not supported by chromosome counts, and it is weakened by the fact that Harder has proved that either a (+) nucleus or a (−) nucleus in a mycelium of *C. sterquilinus* can be caused to give rise to both (+) and (−) nuclei.

Harder (1926), by microsurgery, isolated the penultimate cell of a growing hypha of *Coprinus sterquilinus* at a time when, owing to the formation of a clamp-connexion between the apical and subterminal cells not having been completed, the cell was uninucleate. Many such uninucleate cells were obtained. These cells were set separately on a nutrient culture medium, whereupon they soon began to grow and form a mycelium. Each mycelium came to have

conjugate pairs of nuclei in its cells, formed clamp-connexions in association with conjugate nuclear divisions and, finally, developed normal fruit-bodies. Harder concluded that: the nuclei of the conjugate pairs are sexually differentiated; a nucleus of the absent sex must be produced by the nucleus of the one sex that was present in the uninucleate cell; and, therefore, the isolated nuclei must have a mixed-sex tendency.

Harder's findings seems to indicate that, in homothallic species of the *Coprinus sterquilinus* type, homothallism is due to the sexual instability of the haploid nuclei present in the basidiospores and young mycelia: an unstable nucleus, when dividing, gives rise to two daughter nuclei, one of which is relatively (+) and the other relatively (−) and thus provision is made for the establishment of pairs of conjugate nuclei and for the production of clamp-connexions.

Homothallic species may be looked upon as sexually degenerate. Their homothallism secures that each individual derived from a single spore is able, without co-operation with any other individual, to reproduce itself; but this is done at the expense of variability, with the result that homothallic species tend to take no part or very little part in the progressive evolution of species and genera. Coprinus, Puccinia, and many other genera, otherwise heterothallic, contain some homothallic species; but, possibly, there is no large genus of the Higher Fungi in which all the species are homothallic and exclusively self-fertile. It is obvious that the evolution of very many genera has been associated with changes in heterothallic species only. Homothallic Hymenomycetes, Uredinales, etc., may be compared with such sexually degenerate Compositae as the Dandelion, *Taraxacum officinale,* in which, owing to diploid parthenogenesis, every seed is set without any co-operation with the pollen grains.

In homothallic species, although natural selfing is the rule, there is the possibility of occasional sexual interaction between different individuals. In species like *Coprinus sterquilinus, Puccinia malvacearum,* and *Pyronema confluens,* such an interaction would seem to be almost, if not quite, impossible. On the other hand, in *Saccharomyces ellipsoideus* and similar yeast species, ascospores of different asci or haploid yeast-cells derived from different ascospores, that by chance have been brought together, may conjugate

with one another. *Neurospora tetrasperma* and similar species, although normally homothallic, by means of their occasionally-produced, unisexual, dwarf-spores are facultatively heterothallic (Shear and Dodge, 1927). In a homothallic species, if sexual interaction takes place occasionally, there is the possibility of progressive evolution and of the production of new races or even species. However, in homothallic species, as compared with heterothallic species, the chances for producing new kinds of fungi are greatly reduced.

Derx (1925) found that *Penicillium luteum* is heterothallic; but Emmons (1935) showed that this species and eleven other species of Penicillium produce ascocarps on mycelia derived from single ascospores. Whether Emmon's results were due to haploidy (*vide* Section V) or to homothallism comparable with that of *Coprinus sterquilinus* remains to be determined.

For the Uredinales, on the basis of a long array of facts, Jackson (1931) has advanced cogent arguments in support of his conclusion that heterothallism, heteroecism, and a long-cycled life-history, as now exhibited by species like *Puccinia graminis,* are primitive characters of the group, and that all the homothallic, autoecious, short-cycled species, such as *P. malvacearum,* have been derived from heterothallic ancestors now extinct or still living.

In the Discomycetes, there can be but little doubt: that the typical primitive form of sexuality is that of heterothallism, as displayed by *Ascobolus magnificus* (Dodge, 1920), *Ascobolus stercorarius* (Dowding, 1931), etc., where the sexual process (as in Hymenomycetes, Uredinales, and *Neurospora sitophila*) can be initiated by the mating of two mycelia of opposite sex; and that the homothallic species, such as *Pyronema confluens,* have been derived from heterothallic ancestors. *P. confluens* has been studied cytologically by Harper, Claussen, Gwynne-Vaughan, Tandy, and others (Kniep, 1928) with the hope of deciding whether, in the sexual process, there is a single nuclear fusion or two successive fusions, and a single reduction of the chromosomes or two reductions; but, unfortunately, since *P. confluens* is homothallic, the controversy cannot be decided for this fungus by means of experiment.

As Dodge (1939, a) has pointed out, those cytologists who believe that in some Discomycetes there are two successive nuclear fusions (one in the oogonium and the other in the ascus) and two

successive reductions of chromosomes in the ascus can prove their case if they can find a heterothallic Discomycete with three pairs of factors that can be inherited independently and then, by crossing, obtain asci in which all the eight haploid ascospores are genotypically different from one another.

Kerl (1937), in regeneration studies made on *Pyronema confluens,* dissected away young antheridia and unfertilised ascogonia and placed them on culture media, whereupon they grew out into mycelia that soon produced antheridia and ascogonia once more. It thus appears that, in *P. confluens,* the "male" nuclei and the "female" nuclei have embodied within themselves the potentialities of both sexes. In heterothallic Ascomycetes such as *Neurospora sitophila, N. crassa,* etc., no such confusion of sexuality exists and the unisexual mycelia derived from the ascospores have so far never been known to alter their sexual powers. Said Lindegren (1936) in respect to *N. crassa:* "no mutations of sex have been recorded in a study of more than 20,000 ascospore cultures."

In heterothallic Hymenomycetes, while sexual stability is the rule, instances are known in which a haploid mycelium derived from a single basidiospore has become bisexual. Kniep (1920), in *Schizophyllum commune,* observed two monosporous mycelia that, when young, reacted as normal haplonts but that, when examined a year later, were (as indicated by their clamp-connexions) in the bisexual condition; and mutations of a similar character have been observed by Vandendries in *Coprinus radians* (1925, a, b) and *C. micaceus* (1927), by Hanna (1925) in *C. lagopus,* and by D. Newton (1926, b) in *C. Rostrupianus.*

Vandendries (1925, a, b; 1927) has suggested that the Hymenomycetes in general are *hetero-homothallic, i.e.* that the mycelia derived from single basidiospores are at first haploid and unisexual and therefore heterothallic and that, sooner or later, these same mycelia become dikaryotic and bisexual (with production of clamp-connexions in species that produce these structures) and therefore homothallic. *Coprinus radians* (Vandendries, 1925, a, b), *C. micaceus* (Vandendries, 1927), and *C. Rostrupianus* (D. Newton, 1926, b) are three species that in a high degree are hetero-homothallic, for many of their haplonts that at first in matings behave unisexually in the course of a few weeks or months become bisexual and develop clamp-connexions. However, it has not been proved

that *all* the haplonts in these species become bisexual, and many of the haplonts may be stable for an indefinite period of time. Hanna (1928) cultivated haploid mycelia of *Coprinus lagopus* for three years without any change in their sexual reactions and he concluded that *C. lagopus* "may be considered as a heterothallic species in which sexual mutations from the haploid to the diploid condition occur but rarely." Kniep (1920, 1922) observed in *Schizophyllum commune* only two mutations from the haploid to the diploid condition. Therefore neither *Coprinus lagopus* nor *Schizophyllum commune* can be considered as regularly hetero-homothallic. Mounce (1929) kept monosporous cultures of *Fomes pinicola* in culture for five years, and at the end of this time they were still in the haploid condition. In view of all these facts, an acceptance of Vandendries's suggestion that all Hymenomycetes are hetero-homothallic does not seem warranted.

In the course of the evolution of the Hymenomycetes and Uredinales, the homothallic species of the *Coprinus sterquilinus* and *Puccinia malvacearum* type may well have been produced from heterothallic ancestors as mutations that differed from the parent species in that their haploid nuclei were sexually unstable instead of sexually stable.

In the Hymenomycetes, on the assumption of sexual instability, we may suppose that there are three classes of species:

I. *Very stable.* Mutations from the haploid to the bisexual state (with pairs of conjugate nuclei and clamp-connexions) rare.

Examples: *Schizophyllum commune* (Kniep, 1920) and *Coprinus lagopus* (Hanna, 1925, 1928).

II. *At first stable, then unstable.* In these species the monosporous mycelia at first, when mated, behave as unisexual haplonts; but later, many of these haplonts, when grown separately, become bisexual (come to have pairs of conjugate nuclei and to develop clamp-connexions).

Examples: *Coprinus radians* (Vandendries, 1925, a, b) and *C. Rostrupianus* (D. Newton, 1926, b).

III. *Completely unstable.* All the haplonts very soon become bisexual (come to have pairs of conjugate nuclei and to develop clamp-connexions).

Examples: *Coprinus sterquilinus* (Mounce, 1921, 1922; Brunswik, 1924), *C. stercorarius* (Mounce, 1921, 1922; Brunswik, 1924), and *C. narcoticus* (Brunswik, 1924; Buller, 1924).

The facts and assumptions embodied in the classification just given tend to support the idea that, in the Hymenomycetes, under conditions not understood, a (+) nucleus or a (–) nucleus, on division, may give rise to two nuclei of which, relatively to one another, one is (+) and the other (–). This perhaps fits in with Hartmann's supposition that every gamete or haploid equivalent has hidden within it a bisexual potency.

It is known from the observations of Kniep and of Hanna that the sexual instability of the species listed in Class I is due to sexual mutation; but, so far as Class II is concerned, no such knowledge is available. No one has obtained a monosporous mycelium of such a fungus as *Coprinus radians,* has proved by matings that it is at first unisexual, has then kept it in culture until it has produced clamp-connexions, has then obtained fruit-bodies from it, and, finally, has analysed the spore progeny. Only when that has been done shall we know whether or not, in passing from the haploid to the diploid condition, a mycelium of *C. radians* is subject to a sexual mutation. It may be that all the spores of the fruit-body would be found to be unisexual and of the same sex as the parental spore. If this proved to be the case, then the sexual instability of the species of Class II would be of a different kind to that of the species of Class I, for it would be due to the establishment of a temporary and not of a heritable difference in sex between the two nuclei of each conjugate pair.

In some heterothallic species of Hymenomycetes, not only the diploid mycelia but also the haploid mycelia produce fruit-bodies (*vide* Section V). In two of these species, *Coprinus lagopus* (Chow, 1934) and *Peniophora ludoviciana* (Biggs, 1938), into each haploid basidium of a fruit-body produced on a haploid mycelium (without conjugate pairs of nuclei and without clamp-connexions) there pass *two nuclei.* The two nuclei in each basidium *fuse together.* Then follow meiosis and the production of four haploid basidiospores. It is known from experiment that, in *Coprinus lagopus* (Hanna, 1928) and *Peniophora ludoviciana* (Biggs, 1938), all of the basidiospores produced by a haploid fruit-

body are of one sex and of the same sex as that of the parental basidiospore. Thus, in the species under discussion, we have clear evidence that in some haploid basidia (those derived from a (+) basidiospore) two (+) nuclei fuse together, and that in other haploid basidia (those derived from a (−) basidiospore) two (−) nuclei fuse together. Biggs concludes that the facts she has brought to light "provide additional evidence of the origin of homothallic from heterothallic ancestors."

Since it has been proved that in haploid basidia of *Coprinus lagopus* and *Peniophora ludoviciana* two nuclei of like sex fuse together, one may perhaps be inclined to argue that the homothallism of such a Coprinus as *C. sterquilinus* is not due to sexual instability, but merely to the fact that the nuclei of each mycelium derived from a haploid basidiospore, although sexually all alike (all (+), all (−), or all neuter), simply come together in each young mycelium in conjugate pairs just as come together the two like nuclei in each young haploid basidium of *C. lagopus* or *Peniophora ludoviciana*. However, against this argument may be set: (1) the known fact of occasional sexual instability in *C. lagopus* (Hanna, 1925, 1928) and *Schizophyllum commune* (Kniep, 1920), and (2) the fact that in the haploid mycelia of *C. lagopus* and *Peniophora ludoviciana* the nuclei of like sex never come together to form conjugate pairs associated with the development of clamp-connexions. In *C. lagopus* and *P. ludoviciana* it is only in the haploid basidia (in dikaryotic fruit-bodies the usual place for nuclear fusion) that two like nuclei become associated in that they fuse together. This fusion is of great interest, but must not necessarily be taken as indicating the manner in which such a homothallic species as *Coprinus sterquilinus* has come into existence.

Psalliota campestris, the Common Mushroom, is perhaps the world's best-known agaric, and its basidially two-spored varieties are now grown in great quantities for human consumption. Endeavours are constantly being made to preserve good varieties and to select still better ones; yet, so far, no one has determined whether *P. campestris* is homothallic or heterothallic. If it were found to be heterothallic, one might proceed with confidence to breed new varieties by crossing; but, if it were found to be homothallic, such a program could not be undertaken.

Lambert (1929) obtained nine mycelia derived from as many

single basidiospores of the "Snow White" variety of the two-spored cultivated form of *Psalliota campestris,* and he placed spawn from them singly and separately in standard shelf beds of composted horse manure. Also, he mated portions of the nine mycelia in all possible ways and set spawn from these combinations in other shelf beds. In all the cultures, *i.e.* in those derived from single basidiospores as well as in those derived from two basidiospores, normal "Snow White" fruit-bodies developed. Unfortunately, this result, while of much interest, does not decide the question of homothallism or heterothallism: for, if *P. campestris* is homothallic, all the mycelia, whether paired or unpaired, should produce fruit-bodies equally well; while, if it is heterothallic, diploid fruit-bodies should be produced on the diploid mycelia, and, in addition, haploid fruit-bodies might be produced on the haploid mycelia.

Klioushnikova (1938), working on the two-spored form of *Psalliota campestris* as cultivated in Russia, like Lambert, obtained fruit-bodies from mycelia derived from single basidiospores. He states that these fruit-bodies "were normal and had formed an abundant quantity of spores" and added "This fact makes the homothallic character of *Psalliota campestris* (the two-spored form) quite evident."

The cultivated form of *Psalliota campestris* may well be homothallic, but Klioushnikova's argument that it actually is homothallic is not invulnerable, for the fruit-bodies derived from his monosporous mycelia may have been haploid like the fruit-bodies produced on the haploid mycelia of various heterothallic Hymenomycetes. It is true that the haploid fruit-bodies of many heterothallic Hymenomycetes, *e.g. Coprinus lagopus,* are less robust and less perfect than the diploid fruit-bodies (*vide infra,* Section V); but, as Kniep (1928) has insisted, in some heterothallic Hymenomycetes the haploid fruit-bodies do not differ in appearance from the diploid.

Cytological observations on *Psalliota campestris* (cultivated form) have been made by various investigators. Hirmer (1920) found that the cells in the stipe and fleshy tissue of the pileus are multinucleate and that the nuclei divide conjugately (as explained in Section II). It was observed by Maire (1902), Sass (1929, b), Colson (1935), and Sarazin (1938) that: two nuclei enter each basidium and there fuse together; the fusion nucleus divides twice; of the four haploid nuclei so produced two migrate into one basidio-

spore and two into the other basidiospore; and the nuclei in the spores often divide before germination so that the spores may become multinucleate (Sass, Sarazin). Buhr (1932) made similar observations; but, in addition, he found that, in some basidia, a single nucleus enters each of the two basidiospores and, thereafter, the other two nuclei in the basidium-body degenerate. Thus, according to Buhr, while most of the basidiospores receive two nuclei, others receive only one. Sass (1936) and Klioushnikova (1938) found that the germ-tubes are multinucleate, and Klioushnikova determined that the number of nuclei in an old mycelium of monosporous origin varies from seven to eleven. In the haploid nuclei Colson (1935) counted nine chromosomes and Sarazin (1938) only four. But all these cytological observations taken together do not permit us to decide the question of homothallism or heterothallism: for, in homothallic species such as *Coprinus sterquilinus*, there is conjugate nuclear division in the mycelium and fruit-bodies resembling that in the diploid mycelium and fruit-bodies of a heterothallic species, *e.g. C. lagopus;* and, in haploid two-spored forms of some heterothallic Mycenae (Smith, 1934) and in the four-spored haploid fruit-bodies of the heterothallic species *Coprinus lagopus* (Chow, 1934) and *Peniophora ludoviciana* (Biggs, 1938), two nuclei enter each basidium and fuse together.

To determine whether *Psalliota campestris* is homothallic or heterothallic is a little difficult owing to the absence of clamp-connexions, but it could be accomplished as follows: Obtain monosporous mycelia and fruit-bodies derived therefrom and, using Hirmer's method (1920), find out whether or not conjugate nuclear division takes place in the hyphae during cell-division. If, under the conditions set forth, conjugate nuclear division does take place, then *P. campestris* resembles *Coprinus sterquilinus* and is homothallic; whereas, if it does not take place, *P. campestris* resembles *C. lagopus* and is heterothallic. In the latter case, confirmation of the conclusion in respect to heterothallism could be obtained by mating monosporous mycelia in all possible ways and then finding that conjugate nuclear division can be observed in the mycelia and fruit-bodies derived from some matings but not in those resulting from certain other matings.

Of course, if two monosporous mycelia derived respectively from two distinct races of the cultivated *Psalliota campestris* were to be

mated side by side and, as a result, a distinctive hybrid race of mushrooms were produced on the culture bed, then, without any cytological investigation whatsoever, we should have clear evidence of sexual interaction and could conclude that *P. campestris* is heterothallic. Up to the present, however, no such evidence of heterothallism has been obtained.

It has been noticed by E. B. Lambert, J. W. Sinden, and A. M. Kligman in the U. S. A. (Lambert, 1938, and personal communication) and by Sarazin (1939) that mycelia of the cultivated form of *Psalliota campestris,* derived from individual basidiospores, often differ greatly in mode of growth on agar, rate of growth on agar and in beds of compost, appearance of sporophores, and yield of sporophores. Hence, without any mating of monosporous mycelia, it is possible to make selections and thereby to attempt to isolate outstanding strains.

IV. THE PATH OF NUCLEAR MIGRATION

In *Coprinus lagopus, Neurospora sitophila, Puccinia graminis,* and other heterothallic Higher Fungi, when two large haploid mycelia of opposite sex are mated, the nuclei derived from a mycelium of one sex often travel a distance of several millimetres or centimetres through the hyphae of the mycelium of opposite sex before they find their conjugate mates. Therefore one may ask: how do the migrating nuclei pass from one cell to the next? how do they get through the numerous septa that would seem to bar their progress?

Lehfeldt (1923) was the first to attempt to answer our question. He investigated mated mycelia of a Hymenomycete, *Typhula erythropus,* in which the nuclei were migrating. He states that, in fixed and stained preparations, he observed a number of septa that had been dissolved on one side and reduced to "three-quarter" or "half" septa, thus permitting nuclei to pass through them; and, in his Text-fig. 1 *a,* he shows individual nuclei drawn-out and, as it were, in the act of threading themselves through the hole in a partially-dissolved septum. He also states that the lateral wall of a hypha at the side of a partially-dissolved septum was sometimes bulged outwards slightly, as if to increase the width of the septal opening and thus to facilitate the movement of a nucleus passing through it. In 1931, I accepted Lehfeldt's solution of the migra-

tion problem but, in 1933, expressed scepticism in regard to it and remarked that his work "needs verification before it can be accepted." In 1937, Noble, in fixed and stained preparations of *Typhula Trifolii*, made observations confirmatory of those of Lehfeldt. But neither Lehfeldt nor Noble studied living mycelia and they do not seem to have been aware that, as Wahrlich (1893) had shown for the Higher Fungi in general including the Clavariaceae, there is an *open pore* in the centre of each septum, so that there is protoplasmic continuity from cell to cell.

The septum in the mycelium of the Fungi in general is formed by an annular ingrowth from the lateral wall of a hypha. In *Rhizopus nigricans* and other Mucoraceae, the septum becomes an imperforate disk; while, in the Higher Fungi, the septum stops its centripetal growth in time to leave an open pore at its centre. The time taken for the formation of a septum, complete with its pore, was found to be: for a Hymenomycete, *Corticium solani*, about 10 minutes; and for a Discomycete, the Ciboria that grows on male Birch catkins, about 6 minutes (Buller, 1933).

In living hyphae of the Higher Fungi, the pore of each septum is open; but, as I showed (1933), if one kills a cell on one side of a septum and leaves the cell on the other side of the septum alive, the pore becomes instantaneously blocked by the formation of a *pore-plug*. This is reminiscent of what happens to the pores of the sieve-tubes of a Flowering Plant; for they, too, under certain conditions, become blocked.

In 1933, I observed in various Pyrenomycetes, Discomycetes, and Hymenomycetes, during the growth of the mycelium, a flow of cytoplasm in a stream from one cell to the next through septum after septum (in *Pyronema confluens* through 161 successive septa) *via* the septal pores; and this inclined me to the view that the migrating nuclei also pass from cell to cell through the septum *via* the septal pore, thus proceeding along the line of least resistance. Recently, Dowding and Buller (1940) have advanced experimental and histological evidence supporting their conclusion that, in the Pyrenomycete *Gelasinospora tetrasperma*, when nuclei of one sex are migrating through the septate hyphae of a mycelium of opposite sex, the septa are not dissolved in the manner described by Lehfeldt for *Typhula erythropus* and the nuclei do actually pass through the septal pores.

Also, as is well known, in the Hymenomycetes, the four basidiospores of a basidium are formed before the four haploid nuclei migrate into them and the migration takes place *via* the extremely narrow necks of the sterigmata. The channels in these necks are no wider than the septal pores of the mycelium.

Thus, in view of the observations of Dowding and Buller on *Gelasinospora tetrasperma* and of the fact that nuclei can pass rapidly through the sterigmatic necks of basidia, we may, at least for the present, accept the view that, in the Higher Fungi, during the initiation of the sexual process, the migrating nuclei pass from cell to cell *via* the septal pores.

V. HAPLOIDY

Haploidy as a mutant condition of the sporophytes of Higher Plants is known in a number of species, *e.g. Datura stramonium,* and *Nicotiana tabacum.* In these plants the haploid sporophytes, as compared with normal diploid sporophytes, are somewhat dwarfed and less vigorous, but otherwise in appearance are nearly normal. Their pollen grains exhibit a high degree of sterility.

Haploidy also occurs in the Hymenomycetes. Thus, in *Coprinus lagopus,* as observed in my laboratory, a unisexual haploid mycelium developed from a single basidiospore may produce a haploid fruit-body bearing spores of the same genetic constitution as the spore from which the mycelium was derived. This was proved by Hanna (1928). He even succeeded in raising as many as ten successive generations of haploid fruit-bodies. Like the haploid as compared with the diploid sporophytes of Higher Plants, the haploid fruit-bodies of *Coprinus lagopus* are dwarfed and less vigorous than normal dikaryotic (diploid) fruit-bodies but develop the usual stipe, pileus, and lamellae. The pilei of normal fruit-bodies of *C. lagopus* are black in appearance owing to the large number of black spores borne on the gills. The lack of vigour and decreased fertility in the haploid fruit-bodies is shown by the fact that many of them do not elongate their stipes, open their pilei, or produce any spores, while those attaining the most complete development produce but relatively few spores so that their pilei are pale in colour.

Kniep (1928) in treating of sex in Lower Plants, has divided apomiktic development, *i.e.* development in the absence of a sexual

process, into (1) *gametic apomixis,* (2) *gametangial apomixis,* and (3) *somatogenous apomixis,* and it is in the last category that he has placed what is here being treated of as haploidy, namely, the development of haploid fruit-bodies of *Coprinus lagopus* and other heterothallic Hymenomycetes.

The fact that in *Coprinus lagopus* haploid fruit-bodies are formed on haploid mycelia affords proof that in this fungus the presence of conjugate nuclei in the hyphae is not necessary for fruit-body development.

Among other Hymenomycetes that are able to develop haploid as well as dikaryotic fruit-bodies are: *Schizophyllum commune* (Wakefield, 1909; Kniep, 1920); *Armillaria mucida, Collybia tuberosa* and *Typhula erythropus* (Kniep, 1922); *C. velutipes* (D. Newton, *vide* Buller, 1924; Zattler, 1924); *Panaeolus campanulatus* (Vandendries, 1923, b); *Coprinus ephemerus* (Brunswik, 1924); *Fomes pinicola* (Mounce, 1929); and *Peniophora ludoviciana* (Biggs, 1938). The haploid fruit-bodies of *Collybia velutipes* (Zattler, 1924) and of *Coprinus ephemerus* (Brunswik, 1924) in their imperfect development and reduced basidiospore-production resemble those of *Coprinus lagopus.* Zattler managed to raise five successive haploid generations of *Collybia velutipes* before the line ceased to produce any more basidiospores. Biggs states that in *Peniophora ludoviciana* haploid fruit-bodies, as compared with diploid, are thinner and appear in culture about a week later. The haploid fruit-bodies of *Fomes pinicola* obtained in culture by Mounce were nothing more than thin brownish crusts having a few daedaloid pores that did not yield any basidiospores.

Lack of vigour is perhaps not necessarily a characteristic of haploid as compared with diploid fruit-bodies; and it would seem that vigorous haploid fruit-bodies are produced, among other species, by *Camarophyllus* (*Hygrophorus*) *virgineus* (Bauch, 1926) and various Mycenae, *e.g. Mycena alkalina* (Smith, 1934).

Among the Hymenomycetes that are unable to develop haploid fruit-bodies is *Coprinus Rostrupianus.* In this species the fruit-bodies develop from large black sclerotia. D. Newton (1926, b) observed in my laboratory that, while sclerotia developed on dikaryotic mycelia gave rise to normal fruit-bodies, sclerotia developed on haploid mycelia never produced any fruit-bodies and, when kept moist, merely grew out into mycelia.

According to Kniep (1928), in *Schizophyllum commune* the young basidia of a haploid fruit-body contain a single haploid nucleus. This divides twice and gives rise to four haploid nuclei resembling the parent nucleus, and then the four nuclei make their way into the four basidiospores. Thus, the four spores of each basidium, and all the spores of the haploid fruit-body, are genotypically alike. Kniep's observations also prove that, in the Hymenomycetes, it is possible for a basidium to attain its normal form even when its development is not associated with nuclear fusion and meiosis.

While in the haploid form of *Schizophyllum commune* (Kniep, 1928) and also in the haploid forms of certain Mycenae, *e.g. Mycena galericulata* (Kühner, 1927), *M. alkalina, M. capillaris, M. citromarginata, M. dissiliens, M. leptocephala, M. polygramma,* var. *albida,* and *M. roseipallens* (Smith, 1934), only one haploid nucleus enters each basidium so that in a basidium there is no nuclear fusion; yet, in the haploid forms of *Coprinus lagopus* (Chow, 1934), *Mycena metata, M. clavicularis, M. graveolens, M. immaculata,* and *M. viscosa* (Smith, 1934), and *Peniophora ludoviciana* (Biggs, 1938), two haploid nuclei enter each basidium and there undergo fusion. In haploid basidia where fusion occurs, just as in haploid basidia where no fusion occurs, the basidiospores developed on the sterigmata are all of one sex, and of the same sex as the parental basidiospore. This, as already recorded in Section III, has been proved experimentally for *Coprinus lagopus* by Hanna (1928) and for *Peniophora ludoviciana* by Biggs (1938). In both these species, the haploid basidia resemble the diploid basidia in that they bear four basidiospores (Chow, 1934; Biggs, 1938).

Haploid fruit-bodies of certain heterothallic Hymenomycetes, as well as dikaryotic (diploid) fruit-bodies, occur under natural conditions in the open. This was indicated by investigations on the nuclear condition of the basidia of bisporous forms of *Hygrophorus conicus, H. ceraceus* (Maire, 1902), and *H. constans* (Kühner, 1926), and it was proved for *Camarophyllus* (*Hygrophorus*) *virgineus* by Bauch (1926). Bauch found four-spored and two-spored forms of *C. virgineus* growing in fairy rings near to one another. Each fairy ring was composed entirely of four-spored fruit-bodies or entirely of two-spored fruit-bodies. In the four-spored form the cells of the fruit-body and of the mycelium attached

to its base bore clamp-connexions and contained conjugate pairs of nuclei; whereas, in the two-spored form the cells of the fruit-body lacked clamp-connexions and were all uninucleate. In a basidium of the four-spored form, two haploid nuclei fused together, and this was followed by meiosis and the passage of the four resulting haploid nuclei up the four sterigmata into the four basidiospores; whereas, in a basidium of the two-spored form, there was at first only one haploid nucleus present so that no fusion was possible, the single haploid nucleus divided once only and, finally, the two haploid daughter-nuclei passed up the sterigmata into the two basidiospores.

Also in the genus Mycena, there are many species that, under natural conditions, produce haploid as well as normal dikaryotic (diploid) fruit-bodies; and twelve such species investigated by Smith (1934) have already been named. The haploid form of a Mycena that produces normal dikaryotic fruit-bodies is usually two-spored. Smith found certain two-spored species, *e.g. Mycena margaritispora* (nuclear fusion in basidium) and *M. lasiosperma* (no nuclear fusion in the basidium), represented by haploid fruit-bodies only, and he concluded that here "we may be dealing with segregated forms of otherwise unknown species." He also remarked that "It is possible that some of these isolated two-spored species may be stable forms which have persisted while the ancestral forms have been lost in the course of evolution."

Although *Coprinus lagopus* can produce haploid fruit-bodies on haploid mycelia, probably it but rarely does so under natural conditions. Very many times, during more than twenty years, fresh horse-dung balls were brought into my laboratory and placed in culture dishes. On these balls fruit-bodies of *C. lagopus* constantly came up; but they were always diploid in appearance and never haploid. This non-production of haploid fruit-bodies can be readily explained as follows. The horse-dung balls, on deposition, contained many *C. lagopus* basidiospores of all the four sexual types, (*AB*), (*ab*), (*Ab*), and (*aB*). The spores germinated and the young mycelia soon came into contact and fused with one another. Mycelia of opposite sex diploidised one another. In *C. lagopus,* diploid mycelia can diploidise haploid mycelia (*vide* Section XXII); and, doubtless, in the dung-balls under discussion, this process assisted in causing general diploidisation of the haploid

mycelia. In the absence of haploid mycelia in the dung-balls in the later stages of mycelial development, on the dung-balls only diploid fruit-bodies could be developed.

Haploidy may also occur in the Uredinales. Kursanov (1922) found in three different places near Moscow a form of *Aecidium punctatum* (a stage of *Tranzschelia pruni-spinosae*) on *Anemone ranunculoides* that differed from the normal form on the same host in that its aecidia were haploid: the aecidiosporophores and aecidiospores all contained single nuclei instead of conjugate pairs of nuclei. Kursanov's observations were made before Craigie (1927, a, b) had shown that Rusts like *Puccinia graminis* are heterothallic. In the light of our present knowledge of sex in the Uredinales, we may interpret Kursanov's form of *A. punctatum* as a manifestation of haploidy in a normally dikaryotic species.

In certain other Rusts uninucleate varieties have become known. Thus Mme Moreau (1911–1915) found *Endophyllum euphorbiae-sylvaticae* var. *uninucleatum* that resembles the parent species except in that the aecidiospores from the first are provided with a single nucleus instead of a pair of conjugate nuclei. Here, again, we can interpret this phenomenon as a case of haploidy in a dikaryotic species. It can be imagined that Mme Moreau's uninucleate variety had developed from a single basidiospore or from several basidiospores of the same sex instead of from a mixture of (+) and (–) basidiospores.

In *Puccinia graminis, P. helianthi,* and many other Rusts, the development of proto-aecidia into uninucleate aecidia, *i.e.* haploidy in aecidial production, has never been observed.

In *Puccinia graminis,* normally, all the uredospores in a uredospore sorus are dikaryotic; but in certain abnormal selfed strains in which uredospores were produced on Barberry leaves, Newton and Johnson (1940) observed on the Barberry leaves some uredospore sori in which the uredospores were all uninucleate and others in which some of the uredospores were uninucleate and others binucleate. Here, again, we have a display of haploidy and, in this instance, in a species well known to be heterothallic and one in which haploidy is not normally exhibited.

The Sporobolomycetes constitute a group of basidiomycetous yeasts. Derx (1930) has distinguished two genera, Sporobolomyces and Bullera, and a number of species. These have a wide

distribution. The Sporobolomycetes develop asymmetrical basidiospores on aerial sterigmata and shoot them away by the drop-excretion mechanism in exactly the same way as do the Hymenomycetes, Uredinales, and Tilletiaceae (Kluyver and van Niel, 1924; Buller, 1933). Guilliermond (1927) and Buller (1933) both found that the life-history of *Sporobolomyces salmonicolor, S. roseus,* etc., is uninucleate throughout. It may well be that we have here another display of haploidy and that, when in a single species like *S. roseus* strains have been obtained from various localities and mated in all possible ways, in some combinations the yeast-cells will fuse and so initiate a dikaryotic phase in the life-history, corresponding to that in *Coprinus lagopus* or *Puccinia graminis.*

VI. A CELL CONTAINING CONJUGATE NUCLEI IS A DIPLOID CELL

The presence of conjugate nuclei in the cells of the mycelia and fructifications of the Hymenomycetes, Rust Fungi, and other Basidiomycetes and in the ascogenous hyphae of Pyrenomycetes, Discomycetes, and other Ascomycetes suggests the following question: Is a cell containing a complete set of pairs of homologous chromosomes so distributed that one chromosome of each pair is enclosed within one nuclear membrane and the other chromosome within another nuclear membrane a haploid cell or a diploid cell?

In so far as the Higher Fungi are concerned, the answer to our question, as given by myself and, directly or by implication, by Kniep (1920, 1928), Vandendries (1923), Craigie (1940) and some others, may be thus expressed: *a cell containing a pair of conjugate nuclei is a diploid cell.*

The reasons for this answer are three: (1) the conjugate nuclei provide the cell in which they lie with a complete set of pairs of homologous chromosomes; (2) the chromosomes of both nuclei affect the cell cytoplasm and influence mycelial development; and (3) certain hybrid mycelia and fructifications containing conjugate nuclei exhibit the Mendelian phenomenon of dominance and recessiveness.

(1) In such a fungus as *Coprinus lagopus* it is obvious that, if we were to count the chromosomes and examine them in detail, we should find *the same number of chromosomes* and exactly *the same series of genes* collectively present in the conjugate nuclei of all the mycelial and fruit-body cells from which any given basidium has

descended as are present in the single diploid nucleus of the basidium immediately after this nucleus has been formed by nuclear fusion.

(2) That in such a Toadstool as *Coprinus lagopus* the chromosomes of both of the nuclei of a conjugate pair affect the cytoplasm and influence mycelial development is clearly indicated by the fact that a haploid mycelium in which each cell contains a haploid nucleus (1) does not produce any clamp-connexions, (2) branches at an obtuse angle, and (3) develops oidiophores and oidia; whereas, after such a mycelium has come to have conjugate nuclei in its cells, it (1) develops clamp-connexions, (2) branches at an acute angle, and (3) ceases to develop oidiophores and oidia (Buller, 1931). For the formation of clamp-connexions, to change the branching of the hyphae from obtuse-angled to acute-angled, and to bring about cessation in the production of oidiophores and oidia both sets of chromosomes, both genoms, are required.

In *Coprinus lagopus,* further evidence that both of the nuclei of a conjugate pair affect the cytoplasm is afforded by a comparison of haploid and dikaryotic fruit-bodies. As we saw in the previous Section, a haploid fruit-body is always much less vigorous and less fertile than a dikaryotic fruit-body. This can readily be explained on the supposition that the haploid set of chromosomes (the genom) of each of the two nuclei of a conjugate pair of nuclei influences fruit-body development.

Goldschmidt (1928) investigated the Smut *Ustilago violacea* and showed that the mating of certain monosporidial races profoundly influences their parasitic powers. Said Christensen and Rodenhiser (1940): "His data indicated that association of two haploid nuclei of different parasitic abilities may have physiologically the same effect as if they had fused to give a diploid stage. The dikaryophytic hybrid produced from crossing monosporidial lines from two different races was capable of attacking the hosts susceptible to both parental races". Thus in the Ustilaginales, just as in the Hymenomycetes, we have evidence that both of the nuclei of a conjugate pair influence the cytoplasm of the cell in which they lie.

The thesis that both of the genoms in the two haploid nuclei of a conjugate pair of nuclei influence the cytoplasm of the cell is further supported by facts observed in the Uredinales in connexion with the crossing of differing varieties of *Puccinia graminis.*

Puccinia graminis secalis attacks Rye but not Wheat, while *P. graminis tritici* attacks Wheat but not Rye. Levine and Cotter (1931) crossed these two varieties and obtained a dikaryotic hybrid rust that could attack varieties of Rye and Wheat rather severely, which neither of the parent rusts could do.

Puccinia graminis tritici attacks Wheat but not Oats, while *P. graminis avenae* attacks Oats but not Wheat. Johnson and Newton (1933) crossed these varieties and obtained a dikaryotic hybrid rust that could attack some Wheat and some Oat varieties, which neither of the parent rusts could do.

(3) In Hymenomycetes and Rust Fungi, when two distinct races of any species are crossed, there comes into existence a *hybrid mycelium* in each of whose cells there is a pair of conjugate nuclei; and of each pair of nuclei one nucleus has been derived from one parent and the other nucleus from the other parent. In such a hybrid mycelium, and in the fructifications that it produces, the phenomenon of Mendelian dominance and recessiveness has been observed, and some particular instances thereof will now be cited.

VII. MENDELIAN DOMINANCE IN HYBRID MYCELIA AND FRUCTIFICATIONS

(a) *Schizophyllum commune* is a wood-destroying agaric having whitish, sessile, fan-shaped pilei with split gills, and it is found in Europe and North America on logs and sticks. Its sexuality was investigated experimentally by Kniep (1920). As usual in Hymenomycetes, its dikaryotic mycelia give rise to dikaryotic fruit-bodies that produce and liberate haploid spores. Kniep showed that *S. commune* is heterothallic.

Zattler (1924) obtained a race of *Schizophyllum commune* that produces abnormal rounded knot-like fruit-bodies (Knäuel-Fruchtkörper) instead of normal dorsi-ventrally flattened fruit-bodies. He called the factor for the production of knot-like fruit-bodies g and the factor for the production of normal fruit-bodies G and, then, in matings and crossings of *haplonts* (haploid mycelia) observed that:

$g \times g$ haplonts gave knot-like fruit-bodies,

$G \times G$ haplonts gave normal fruit-bodies, and

$G \times g$ haplonts gave *normal* fruit-bodies.

From these results and further studies made with the haploid

progeny of the $G \times g$ fruit-bodies Zattler rightly concluded that the factor G carried in one nucleus of a conjugate pair is dominant over the factor g carried in the other nucleus of the conjugate pair.

(b) *Collybia velutipes* is an agaric with a reddish-yellow pileus and a densely velvety blackish-brown stipe. It is found on stumps, logs, and roots as well as on the bark of living trees. It is heterothallic.

Zattler (1924) obtained races of *Collybia velutipes* differing in colour and, after making the required matings, found that the development of colour in the dikaryotic mycelium produced by mating haplonts (haploid mycelia) is dependent on the presence in the nuclei of two allelomorphic pairs of factors, *Rr* and *Vv*. He isolated four kinds of haplonts that could be used for matings: *RV* haplonts, deep brown; *Rv* and *rV* haplonts, of two differing intensities of bright brown; and *rv* haplonts, pure white without any trace of colour. On mating these haplonts in all possible ways, Zattler obtained dikaryotic mycelia; and a study of these mycelia led him to the conclusion: that the factors R and V both cause browning of the mycelia; that the factors r and v do not cause browning; and that the factor R is dominant over the factor r, and the factor V dominant over the factor v. Finally, Zattler remarked that, as shown by his experiments, "for the display of dominance the karyogamy of conjugate nuclei is not necessary."

(c) *Panus stypticus* is yet another heterothallic wood-destroying agaric. Its fruit-bodies are found in small clusters on stumps or branches of Birches.

The writer (1924) discovered that, in *Panus stypticus,* there are two physiologically different races: a North-American race, *P. stypt. luminescens,* that emits light from its mycelia and fruit-bodies; and another race, found in England, France, and Germany, *P. stypt. non-luminescens,* that gives out no light whatsoever. Macrae (1937) crossed the two races and found that the hybrid dikaryotic mycelia are always luminous. Evidently, in each cell of a hybrid mycelium a factor for luminosity carried by the nucleus derived from the luminous parent is dominant over a factor for non-luminosity carried by the nucleus derived from the non-luminous parent.

Macrae (1941) germinated several hundred basidiospores obtained from her hybrid fruit-bodies and found that about half of

them yielded luminous mycelia and the other half non-luminous mycelia. This indicates that in a basidium, during meiosis, segregation takes place in such a way that the basidium comes to bear two basidiospores with the luminous factor and two with the non-luminous factor.

(d) *Coprinus lagopus* of myself and my pupils is considered by Bensaude and myself (Buller, 1931) to be identical with *Coprinus fimetarius* of Bensaude and Quintanilha.

Quintanilha and Balle (1938, 1940) studied the phenomenon of nanism in *Coprinus lagopus* (their *C. fimetarius*). They obtained from certain basidiospores *dwarf mycelia* that, by comparison with *normal mycelia,* grew very slowly and irregularly and never attained to a large size. It was found that the dwarfism of these dwarf mycelia was heritable in a Mendelian manner. To the pair of factors involved the symbol (Nn) was given, (N) being held responsible for normal growth and (n), a sublethal allelomorph, responsible for the incapacity to develop shown in the phenomenon of nanism.

The combinations $(N) \times (N)$ haplonts and $(N) \times (n)$ haplonts both yielded normal dikaryotic mycelia that bore the usual clamp-connexions, grew well, and fruited in due course, thus indicating that the factor for normal growth (N) held in one of the nuclei of each conjugate pair is dominant over the factor for nanism (n) held in the other nucleus of the pair.

The combination $(n) \times (n)$ haplonts resulted in continued nanism without any production of clamp-connexions or of fruit-bodies.

Quintanilha and Balle studied the progeny of the dikaryotic fruit-bodies produced by an $(N) \times (n)$ combination. They removed and succeeded in germinating all of the four spores of 101 basidia. It was then found that of the four spores of each basidium two gave rise to normal mycelia and two to dwarf mycelia. Thus it was proved that, during meiosis of the fusion nucleus in each of the 101 hybrid basidia, Mendelian segregation had taken place in such a way as to give rise to two haploid nuclei bearing the dominant factor (N) and two haploid nuclei bearing the recessive factor (n).

(e) *Peniophora Allescheri* is a heterothallic Hymenomycete that forms flat crusts on dead branches of trees. Nobles (1935) ob-

served that normally in this species: (1) oedocephaloid conidiophores are produced on both the haploid and the dikaryotic mycelia; (2) the conidia are all uninucleate; and (3) the uninucleate conidia on a dikaryotic mycelium give rise to haploid mycelia of both sexes, some mycelia being (+) and others (–).

Nobles obtained a mutant haplont of *Peniophora Allescheri.* A normal haplont of *P. Allescheri* is (1) slow-growing with scant aerial mycelium and (2) bears conidiophores; whereas the mutant haplont (1) grows rapidly with abundant cottony aerial mycelium and (2) does not bear conidiophores. The combination, normal haplont × mutant haplont, yielded a dikaryotic hybrid mycelium that (1) grew rapidly with abundant aerial mycelium like the mutant parent and (2) bore conidiophores and conidia like those of the normal parent. As was to be expected, of the uninucleate conidia produced on the conidiophores of a hybrid dikaryotic mycelium some gave rise to haplonts like the normal parent, while others gave rise to haplonts like the mutant parent.

Nobles concluded that, in crosses like the one she had studied, "both of the nuclei" of a conjugate pair "are effective in determining the nature of the resulting diplont". It is also clear that, in a hybrid dikaryotic mycelium of *Peniophora Allescheri* produced by crossing a normal haplont with the mutant haplont: rapid growth (R) is dominant over slow growth (r); and conidiophore production (C) is dominant over non-production of conidia (c).

(f) *Puccinia graminis tritici* causes the Black Stem Rust disease of wheat. Its genetics has been studied in considerable detail by Newton and Johnson. These investigators found races differing in colour (1927). Crossing of these races on Barberry bushes by means of the nectar-mixing technique enabled Newton and Johnson to obtain dikaryotic hybrid aecidiospores. These aecidiospores were then sown on seedling wheat plants. Here they gave rise to a dikaryotic mycelium that produced pustules of dikaryotic uredospores. Normally, the uredospores of *P. graminis* are *red;* and the red appearance is due to a *yellow-orange* carotinoid pigment present in the cytoplasm and an unrelated *greyish-brown* pigment that impregnates the cell-wall. From breeding experiments the mode of inheritance of these two pigments was elucidated (Newton, Johnson, and Brown, 1930; Johnson, Newton and Brown, 1934). The pair of allelomorphs for cytoplasm colour was called

Yy, and the pair of allelomorphs for cell-wall colour *Gg*. On crossing haplonts differing in colour, four colour-forms of pustules of dikaryotic uredospores were obtained to which symbols could be attached: (1) red, *YyGg;* (2) orange, *Yygg;* (3) grey-brown, *yyGg;* and (4) white, *yygg*. It was concluded: that the factor *Y* causes the production of colour in the cytoplasm; that the factor *G* causes the production of colour in the cell-wall; that the factors *y* and *g* do not cause colouration; and that *Y* is dominant over *y* and *G* dominant over *g*.

(g) Newton, Johnson, and Brown (1930), Johnson, Newton, and Brown (1934), and Johnson and Newton (1940) crossed races of *Puccinia graminis tritici* differing in pathogenicity; and here again, in some crosses, the phenomenon of dominance and recessiveness was encountered. The details of one of their experiments will now be recorded.

Johnson and Newton (1940, b), in their work on *Puccinia graminis tritici,* obtained pathogenically homozygous mycelia of Race 9 and Race 36. They tested the pathogenicity of these races on twelve differential wheat host-seedlings selected by Stakman and Levine for differentiating races of wheat stem-rust; and it was found that, as usual, *Kanred* (a winter wheat) is not attacked by Race 9, whereas it is heavily attacked, with the production of large uredospore pustules, by Race 36.

The teleutospores of Race 9 and Race 36 were germinated and the resulting basidiospores (sporidia) were sown separately on different Barberry bushes, and thus haploid pustules of both races were obtained. By means of the nectar-mixing technique the two races were then crossed. The combination Race 9 × Race 36 yielded hybrid dikaryotic aecidia and aecidiospores. These hybrid aecidiospores were then sown on Little Club wheat, so that an abundance of dikaryotic hybrid uredospores became available. These uredospores were now sown on the twelve differential hosts, and it was then observed that on *Kanred* there was *no infection.* This indicated clearly that on this host Race 9 is dominant over Race 36. In other words, in the dikaryotic hybrid mycelia a factor for non-pathogenicity carried by the nucleus derived from Race 9 is dominant over a factor for pathogenicity carried by the nucleus derived from Race 36.

The F_1 hybrid mycelia formed by crossing Race 9 and Race 36

developed teleutospores and these, on germination, gave rise to basidiospores. The basidiospores were sown on Barberry bushes and thus haploid pustules were obtained. The nectar of these (+) and (−) pustules was mixed and, thereafter, aecidia and aecidiospores developed. Then from a single aecidium of each of the many aecidial pustules aecidiospores were removed in a mass and sown on a separate Little Club host. Thus numerous F_2 dikaryotic uredospore pustules and uredospores were obtained. The uredospores derived from each original sowing of aecidiospores were then transferred to the twelve differential wheat host-seedlings. It was then found that on *Kanred* there was in some transfers *no infection* whatsoever, while in other transfers there was *heavy infection.* The ratio of non-infected to infected Kanred host-plants was found to be about 3:1, which indicates that the pathogenicity had been inherited in a Mendelian manner.

(h) The Septobasidiales (Couch, 1938) constitute a group of Basidiomycetes made up of numerous species that live symbiotically in association with scale insects on the bark of living trees. In the course of his long-continued study of the group, Couch observed two naturally-occurring interspecific hybrids. One of these, when examined, was at once recognised as having been derived from a natural cross between *Septobasidium pseudopedicellatum* and *S. Schweinitzii.* This hybrid displays dominance in the production of its dikaryotic probasidia and, therefore, it will now be described.

Septobasidium pseudopedicellatum and *S. Schweinitzii* occur together on the same branches of *Fraxinus americanus,* are associated with the same species of scale insect (Chionaspis sp.), and produce basidia and basidiospores at the same time. Infection of the insects probably occurs, as in *S. Burtii,* while the young insects are crawling over the sporulating outer surface of the mycelial mat. The hybrid about to be described was probably produced as the result of anastomoses of bud cells in an insect infected by the spores of both the fungus species. We may assume that the hybrid mycelium contains pairs of conjugate nuclei, and that nuclear fusion occurs in the probasidium just as in the teleutospores of the Rust Fungi and in the chlamydospores of the Smut Fungi.

Septobasidium pseudopedicellatum (1) produces "pillars" on the subicular layer of its mycelial mat, (2) has a rounded or oval thick-walled probasidium, and (3) develops from the probasidium

a straight basidium with short sterigmata; whereas *S. Schweinitzii* (1) produces "tents" on its subicular layer, (2) is devoid of a probasidium, and (3) develops a coiled basidium with long sterigmata.

The dikaryotic mycelium of the natural hybrid formed from the combination *Septobasidium pseudopedicellatum* × *S. Schweinitzii,* as observed by Couch, (1) produces on its subicular layer pillars like those of *S. pseudopedicellatum* and tents like those of *S. Schweinitzii,* (2) has a probasidium like that of *S. pseudopedicellatum,* and (3) develops from the probasidium a coiled basidium like that of *S. Schweinitzii.* This last feature clearly indicates that the hybrid under discussion is not a mere chimaeric mixture of the mycelia of the two species involved, but is truly the result of an interspecific cross.

The probasidium of the hybrid just described is formed while the probasidium is still dikaryotic and before nuclear fusion has taken place. Thus it appears that a factor for production of a probasidium present in a nucleus derived from *Septobasidium pseudopedicellatum* is dominant over a factor for non-production of a probasidium present in a nucleus derived from *S. Schweinitzii.*

In the eight cases of hybridity already treated of, the dikaryotic mycelia, fruit-bodies, spores, or probasidium exhibit the phenomenon of Mendelian dominance and recessiveness just as clearly as do the diploid hybrids of so many Flowering Plants and animals.

Naturally-occurring interspecific hybrids are unknown in the Hymenomycetes, Uredinales, Pyrenomycetes, and Discomycetes; but, as we have seen, two have been recorded in the Septobasidiales. In the Ustilaginales, also, natural hybrids between certain species have been found, and an example from this group will now be cited.

Ustilago hordei, a covered smut, and *U. nigra,* a loose smut, both parasitise Barley. *U. nigra* was discovered by Tapke (1932, 1935) and recognised by him as being distinct from the loose smut *U. nuda.*

Ustilago hordei (1) produces a covered type of smut head, (2) develops sporidia on its promycelium (basidium) that infect seedlings, and (3) has smooth chlamydospores; whereas *U. nigra* (1) produces a loose type of smut head, (2) as in *U. hordei,* develops sporidia on its promycelium that infect seedlings, and (3) has echinulate chlamydospores.

Naturally-occurring smuts have been found by Ruttle (1934) that appear to be hybrids between *Ustilago hordei* and *U. nigra;* for they

produce (1) an intermediate type of smut head, (2) as in both species, sporidia on the promycelium, and (3) chlamydospores with varying degrees of echinulation. Thus in these hybrids the characters (1) and (3) are more or less intermediate between those of the parental species and there is no clear-cut display of dominance and recessiveness.

Interspecific hybrids were produced experimentally in the Ascomycetes for the first time by Shear and Dodge in 1927. These workers obtained perithecia with ripe ascospores in the following crosses between species of Neurospora: *N. sitophila* × *N. crassa; N. sitophila* × *N. tetrasperma* (haplonts derived from dwarf-spores); and *N. crassa* × *N. tetrasperma.*

Interspecific hybrids, and even intergeneric hybrids, have also been produced experimentally in the Saccharomycetales. Winge and Laustsen (1938, 1939, b), taking advantage of the fact that the ascospores in the asci of Saccharomyces and Zygosaccharomyces species are unisexual and can act as gametes, with the help of a micromanipulator brought together in a hanging drop of culture medium two ascospores, one derived from one race or species and the other from another race or species. The spores fused with one another and karyogamy followed. The hybrid zygote then germinated and gave rise to a colony of vegetative yeast-cells. In this way Winge and Laustsen produced fourteen yeast hybrids containing diploid nuclei. Of these hybrids the following are examples: *S. Italicus* × *S. validus; S. cerevisiae* (Danish baking yeast) × *S. validus; S. cerevisiae* Race II × Danish baking yeast; *S. cerevisiae* Race II × *Z. Priorianus;* and *S. Mandschuricus* × *S. spec.* 769. One of the hybrids produced from the combination, Danish baking yeast × *S. cerevisiae* Race II, showed advantages over its parents in having a very high production of dry matter and of carbon dioxide combined with good sedimentation and is now being used by a British firm that sells baking yeast (W. and L. 1939, b). Thus by hybridisation it is possible to breed better commercial yeast types.

In field and laboratory studies of the Hymenomycetes neither I myself nor any one else has seen anything so far that would suggest that in this group dikaryotic species-hybrids exist. In general, the species of Hymenomycetes remain morphologically distinct from one another without intermediate forms. Thus on unsterilised horse dung in laboratory cultures several species of Coprinus, *e.g.*

C. lagopus, C. niveus, C. curtus, C. ephemerus, C. Friesii, (all of which are heterothallic) often come up close together, yet their fruit-bodies remain specifically distinct and never show appearances that suggest that the haploid mycelia of two distinct species have given rise to a hybrid dikaryotic mycelium which in turn has given rise to a hybrid dikaryotic fruit-body (Buller, 1933). Brunswik (1924) failed in attempts to cross Coprinus species and Kniep (1928) reports that he made a great number of attempts to cross species in the genera Hypholoma, Collybia, and Mycena, but without success. Vandendries (1923) states that, on pairing haploid mycelia of *Panaeolus campanulatus* and *P. fimicola,* he obtained in one instance a mycelium with clamp-connexions; but the mycelium did not grow well and did not fruit, so that it was impossible to obtain analytic evidence of its hybrid origin. Thus, in the Hymenomycetes, as yet, interspecific hybrids corresponding to those obtained by Dodge in Neurospora are unknown.

In Hymenomycetes and Rust Fungi, as we have seen, the phenomenon of dominance has been observed in hybrid mycelia, fruit-bodies, and uredospores, all of which are dikaryotic. It could not, of course, be manifested in a haploid mycelium or a haploid spore, for in such structures the nuclei are haploid and unpaired. However, there are still other structures in which dominance might be found: in Hymenomycetes, the *basidium;* and in Uredinales, *e.g. Puccinia graminis,* the *probasidium* (teleutospore) and its *basidia.* After the diploid nucleus of a basidium or probasidium has come into existence by nuclear fusion, the basidium continues its development or starts to develop and produces, typically, four sterigmata and four basidiospores. Meanwhile, the diploid nucleus undergoes meiosis with the formation of four haploid unisexual nuclei. These four nuclei pass up the four sterigmata into the four spores, and thus each basidiospore becomes a haploid cell. With the ripening and violent discharge of the basidiospores the development of the basidium comes to an end. One can imagine that the basidia of a Hymenomycete or the teleutospores and basidia of a Rust Fungus produced in a fructification developed from a hybrid mycelium might be so influenced by their diploid nuclei that in the course of their development they would display dominance of one factor or another. No cases of such dominance have been recorded in the Hymenomycetes, but one is known for the Uredinales. This will now be set forth.

The teleutospores of *Puccinia graminis* are normally dark-brown and almost black. This is due to their wall pigment. In connexion with the experiments on uredospore colour made by Newton, Johnson, and Brown (1930) and already recorded under (f), it was found that in some hybrids the teleutospores had the normal *dark-brown* colour, whereas in other hybrids the teleutospores were *pale-brown.* It is to be supposed that the diploid nuclei of the dark-brown teleutospores carried the factors *GG* or *Gg* and that those of the abnormal pale-brown teleutospores carried the factors *gg*. Thus in the teleutospores containing diploid nuclei, just as in the uredospores containing conjugate pairs of haploid nuclei, the factor *G* for cell-wall colour was dominant over the factor *g*.

The fact that, in *Puccinia graminis,* the factor *G* for cell-wall colour is dominant over a factor *g* for lack of cell-wall colour not only in synkaryotic teleutospores but also in dikaryotic uredospores affords direct and conclusive proof that, at least in one case, the two sets of chromosomes in a pair of conjugate nuclei, $(n)+(n)$, in their effect on the development of a particular character, are truly equivalent to the two sets of chromosomes in a single diploid nucleus, $(2n)$.

In the Ustilaginales dominance may be displayed in the mycelium and in the chlamydospores and basidia. A case concerned with the wall markings of the chlamydospores may now be cited. Holton (1931) and Popp and Hanna (1935) found that, on crossing *Ustilago avenae* which has echinulate spores with *U. levis* which has smooth spores, the resulting F_1 chlamydospores were *echinulate.* It was thus shown that echinulation is dominant over smoothness. The ratio of echinulate to smooth chlamydospores in the F_2 generation was 3:1, which indicated that a single factor difference was involved.

Chilton (1938) found that, in *Ustilago zeae,* a high percentage of the chlamydospores produced as a result of crossing certain lines germinated abnormally and their promycelia (basidia) usually disintegrated prior to the formation of sporidia. It was also noticed (Christensen and Rodenheiser, 1940) that the promycelia that produced sporidia were atypical in that their cells were large and irregular. A few of the sporidia grew well, while others budded a few times and then failed to develop any further. Laskaris (1939) obtained similar results with certain inbred crosses of *Spacelotheca sorghi* (Kernel smut of Sorghum). The chlamydospores, pro-

mycelia, and sporidia in certain of his crosses were significantly larger than chlamydospores from normal crosses. Evidently, in both *U. zeae* and *S. sorghi,* lethal factors carried by the diploid nucleus of the chlamydospore may be dominant over factors for normal development and thus cause the promycelium to undergo lysis or an early cessation of development. The observations of Chilton and Laskaris on Smut Fungi are comparable with those made by Dodge on the lethal factors that hinder development in the asci of species of Neurospora.

Flor (1932) and Hanna (1932) succeeded in crossing *Tilletia tritici* having echinulate chlamydospores with *T. laevis* having smooth chlamydospores. In the hybrid bunt-balls the chlamydospores were *smooth,* thus indicating that in the young chlamydospores, after the diploid nucleus has been formed, a factor for smoothness of the wall is dominant over a factor for echinulation. This is the reverse of what was found by Holton and by Popp and Hanna for the hybrids resulting from crosses between *Ustilago avenae* and *U. levis.*

Using pure cultures made from single secondary haploid conidia, Hanna (1932) crossed *Tilletia laevis* which possessed trimethylamine with a strain of *T. tritici* which lacked trimethylamine. The first-generation hybrid chlamydospores resulting from the cross were not only smooth, but they also emitted an odour of trimethylamine. In this cross, therefore, the factors for smooth spore-wall and odour, carried by chromosomes of the diploid nucleus, were both dominant.

In the Septobasidiales, as in the allied groups of the Hymenomycetes, Uredinales, and Ustilaginales, dominance of one character over another may occur in the dikaryotic mycelium and also in the probasidium and basidium after the two conjugate nuclei have fused to form a diploid nucleus. In the natural hybrid *Septobasidium pseudopedicellatum* × *S. Schweinitzii,* as we have seen under (*h*), a factor for production of a probasidium carried in one nucleus of a conjugate pair is dominant over a factor for the non-production of a probasidium carried in the other nucleus of the conjugate pair. In the same hybrid, as we have also seen, after the formation of the diploid nucleus in the probasidium, the basidium produced by the probasidium is always coiled as in *S. Schweinitzii* and never straight as in *S. pseudopedicellatum.* Here, therefore, in the dip-

loid nucleus a factor for the production of a coiled basidium is dominant over a factor for the production of a straight basidium.

In heterothallic Pyrenomycetes and Discomycetes, after the crossing of two distinct races, dominance of one character over another may be realized in a perithecium: (1) during the development of the dikaryotic ascogenous hyphae, *i.e.* hyphae that correspond in nuclear content with the dikaryotic hyphae of mycelia of the Hymenomycetes and Rust Fungi: and (2) during the development of the ascus.

(1) In studies of inheritance in Pyrenomycetes and Discomycetes, no cases of dominance during the development of the ascogenous hyphae have been recognised. They might show in increase or decrease in the number of asci ultimately produced, in failure of the hyphae to produce asci, in pigmentation, and so forth, and perhaps such cases will be revealed when hybrid perithecia are further studied.

(2) In the Pyrenomycete, *Neurospora tetrasperma,* Dodge (1934–1939), has discovered three kinds of lethal factors that prevent the normal development of the ascus. Thus, for example, he found that a lethal factor (*I*) for induration and ascus abortion is dominant over a recessive factor (*i*) for normal ascus development.

In the life-history of species of Saccharomycetales having a sexual process, as shown by Guilliermond (1937) and others, there are haploid cells in the haplophase and diploid cells containing diploid nuclei in the diplophase but, normally, no cells containing dikaryons that multiply by conjugate nuclear division. Therefore, in the Saccharomycetales, it is only in cells containing diploid nuclei that dominance and recessiveness of characters can be exhibited.

As we have seen, Winge and Laustsen (1938, 1939, b) have produced experimentally a number of interspecific hybrid yeasts by crossing species of Saccharomycetes with one another, and an intergeneric hybrid by crossing a species of Saccharomycetes with a species of Zygosaccharomyces. The first-generation hybrid vegetative yeast-cells in the giant-colonies each contain a diploid nucleus with two genoms, one derived from one parent and the other from the other parent (W. and L. 1939, b). Winge and Laustsen (1939, b) found that in their hybrids, the ability to produce a definite enzyme is dominant over lack of such ability and that this is true for saccharase, raffinase, and melibiase. If a yeast type with the

ability to form one of these enzymes is hybridised with another yeast lacking this ability, the hybrid always possesses the ability to form the enzyme.

Also, in the normal yeast-cells of *Saccharomycodes Ludwigii,* each of which contains a diploid nucleus, Winge and Laustsen (1939, a) observed that a factor for normal growth is dominant over a factor for very limited growth, and a factor for the production of short oval cells is dominant over a factor for the production of long cells (*vide* Section III).

From the data set forth in this Section we may conclude that, in the Higher Fungi, the Mendelian phenomenon of dominance and recessiveness can be displayed not only in probasidia, basidia, and asci that contain a diploid nucleus, but also in the dikaryotic mycelia and fructifications that are developed prior to the formation of those structures.

VIII. A NEW DEFINITION OF A DIPLOID CELL

The discovery that conjugate pairs of nuclei are present in the cells of the mycelia and fruit-bodies of Basidiomycetes and in the ascogenous hyphae of the Ascomycetes, together with the further discovery that both of the nuclei of each conjugate pair influence the development of the structures in which they lie, makes it necessary to re-define the term *diploid cell;* and this will now be attempted.

A *diploid cell* is a cell that *contains a complete set of pairs of homologous chromosomes,* half the chromosomes having been derived from one parent and the other half from the other parent.

It will be noted that the new definition of a diploid cell implies the presence in the cell of one or more nuclei, but does not specify their number. In this it differs from the old definition which stated that a diploid cell contains a diploid nucleus.

Diploid cells, as now newly defined, are divisible into two kinds: (1) *dikaryotic* or $(n)+(n)$, and (2) *synkaryotic* or $(2n)$.

(1) A *dikaryotic diploid cell* is a cell that contains two nuclei, $(n)+(n)$, one derived from one parent and the other from the other parent.

Examples: the ova of animals, Mosses, Ferns, and Flowering Plants after a male nucleus has entered and before nuclear fusion has taken place; the cells of the secondary mycelium (a diploidised mycelium or diploid mycelium) and of the normal fructifications of Mushrooms and Toadstools and of Rust Fungi; the aecidiospores,

uredospores, and the cells of young teleutospores of the Rust Fungi; the cells of the ascogenous hyphae of Ascomycetes; young basidia of the Basidiomycetes, and young asci of the Pyrenomycetes and Discomycetes before the two conjugate nuclei have fused; and young zygotes of the Saccharomycetales before the nuclei of the conjugating cells have fused.

(2) A *synkaryotic diploid cell* is a cell that contains a single diploid nucleus, ($2n$), formed by, or derived from a nucleus resulting from, the fusion of two parental nuclei.

Examples: the ova of animals, Mosses, Ferns, and Flowering Plants immediately after the fusion of the male and female nuclei; cells of the sporophytes of Mosses, Ferns, and Flowering Plants; cells of the bodies of animals; young basidia of the Basidiomycetes and young asci of the Pyrenomycetes and Discomycetes immediately after the fusion of the two conjugate nuclei; mature zygotes of the Saccharomycetales after the nuclei of the conjugating cells have fused; and the normal vegetative diploid yeast-cells of *Saccharomyces ellipsoideus, S. cerevisiae,* and other Saccharomyces species.

Since it is known that, normally, all diploid mycelia, fruit-bodies, and ascogenous hyphae in the Higher Fungi are composed of dikaryotic diploid cells, in treating of the Higher Fungi the terms *diploid cell, diploid mycelium, diploid fruit-body, diploid ascogenous hypha* imply that the structures spoken of or written about are dikaryotic. Thus, in practice, it can be but rarely necessary to use the longer terms *dikaryotic diploid cell* or *mycelium,* etc.

Similarly, since it is known that, normally, the sporophytes of Mosses, Ferns, and Flowering Plants and the somatic tissues of animal bodies are composed of synkaryotic diploid cells, in treating of Mosses, Ferns, Flowering Plants, and animals the terms *diploid cell, diploid tissue,* etc., imply that the structures referred to are *synkaryotic.* Thus, in practice, it cannot often be necessary to use the longer terms *synkaryotic diploid cell* or *tissue,* etc.

Normally, in the Ustilaginales (Smut Fungi), the diploid nucleus formed by the fusion of two conjugate nuclei in the young chlamydospore undergoes meiosis in the chlamydospore or in the basidium (promycelium) and reduction in chromosome number and segregation take place in the first or second nuclear division, so that the basidiospores (sporidia) ultimately produced are haploid (Christensen and Rodenhiser, 1940). However, in *Ustilago zeae,* it appears

that, occasionally, no reduction of chromosome number or segregation of sex factors occurs in a whole generation. In fact J. J. Christensen (1931) found that in *U. zeae* cases in which there was no reduction of sex factors for three successive chlamydospore generations. The sporidia were "diploid" and *solopathogenic, i.e.* after inoculation into the host, capable of causing infection and forming chlamydospores.

Applying the new terminology, we may say that, in *Ustilago zeae:* in normal generations the cells of the diploid mycelium are *dikaryotic;* while in the abnormal generations investigated by Christensen the cells of the diploid mycelium are *synkaryotic.*

(Part II of this article will appear in the August issue of the Review.)

THE BOTANICAL REVIEW

Vol. VII August, 1941 No. 8

THE DIPLOID CELL AND THE DIPLOIDISATION PROCESS IN PLANTS AND ANIMALS, WITH SPECIAL REFERENCE TO THE HIGHER FUNGI

A. H. REGINALD BULLER
Professor Emeritus, University of Manitoba

Part II

IX. DISCUSSION OF VIEWS HELD BY B. O. DODGE, AND THE CLASSIFICATION OF HYBRIDS

Dodge, whose studies of *Ascobolus magnificus, Neurospora sitophila, N. tetrasperma, Pleurage anserina, Gelasinospora tetrasperma,* etc., have increased so greatly our knowledge of sex and inheritance in the Ascomycetes, has recently objected to the interpretations that have been placed by students of sex and inheritance in the Hymenomycetes and Uredinales on their experimental results. Since Dodge's objections conflict diametrically with much that has been said in Sections VI, VII, and VIII, an attempt to rebut them must now be made.

Under the heading *"Hybrid" Mushrooms* Dodge (1939) makes the following remarks: "Students have stated that they have obtained hybrids by uniting differently colored haploid races, say, pink and white of opposite sex, through anastomoses, to form a dikaryophytic growth of binucleate cells. Three questions arise here. (1) Are the cells of the dikaryophyte diploid? (2) Is the dikaryophyte heterozygous? (3) Is pink dominant over white? To all these questions the answer is no. A diploid cell, as defined by Strasburger, is one in which its nucleus contains the double number of chromosomes. Such a diploid cell is to be clearly distinguished from a cell containing two haploid nuclei even though they are of opposite sex. Since only diploid nuclei can be heterozygous, pink could not be dominant over white. We cannot think of a sperm as being heterozygous. The fertilized egg could be heterozygous. The

basidia of the mushroom are diploid and normally they are the only cells in connection with which dominance could be expressed."

Already, Johnson and Newton (1940, b), on the basis of observations made on hybrid dikaryotic mycelia and uredospores of *Puccinia graminis,* have rejected Dodge's assertion that dominance cannot be shown in such structures; and, as we have seen in Section VII, cases involving dominance and recessiveness in the dikaryotic mycelia and fruit-bodies of Hymenomycetes have been recorded by various experimentalists.

To all of Dodge's three questions to which his answer is *no,* my answer is *yes.* Already in Sections VI and VII the reasons for regarding a cell containing a pair of conjugate nuclei as diploid and not haploid have been fully set forth. It has been shown that, *collectively,* the two haploid nuclei in a dikaryotic cell do, as a matter of fact, influence the cytoplasm of the cell in the same way as does a diploid nucleus in the body of an animal or in the sporophyte of a Moss, Fern, or Flowering Plant.

Dodge's plea that a cell containing a single diploid nucleus and a cell containing two haploid nuclei of opposite sex are to be distinguished from one another is just, and this plea has been met by my new definition of a diploid cell and by dividing diploid cells into those that are *synkaryotic* and those that are *dikaryotic.*

Certainly, a hybrid dikaryotic mycelium or fruit-body of a mushroom can be heterozygous. Thus, for instance, as shown in Section VII, Zattler (1924) obtained heterozygous fruit-bodies of *Schizophyllum commune* when he crossed a race with normal fruit-bodies with a race with knot-like fruit-bodies. It makes no difference whether the two homologous chromosomes on which heterozygosity depends are both together within one nuclear membrane or whether they are separated within two nuclear membranes, so long as they are in one and the same cell. The thing that matters is the presence of the pair of homologous chromosomes in the cell and not whether they are enclosed in one nucleus or two.

Certainly "pink can be dominant over white," and this kind of dominance has been recognized by workers (Zattler, Johnson and Newton, etc.) whose experiments have been described in Section VII.

In Section I it was shown that Strasburger introduced the terms haploid and diploid to describe the gametophytic and sporophytic

generations of plants (and the corresponding generations of animals) respectively; but, it seems uncertain whether or not he ever expressly defined a diploid cell. However that may be, the definition of a diploid cell ascribed by Dodge to Strasburger was in practice generally accepted by Strasburger's successors.

The old definition of a diploid cell as a cell in which "the nucleus contains the double numbers of chromosomes" was conceived of by students of Mosses, Ferns, and Flowering Plants before the origin of the pairs of conjugate nuclei in the mycelium and fructifications of the Basidiomycetes and Ascomycetes had been elucidated and before the effect of these pairs of nuclei on development had been determined by experiment. Hence the necessity for the new definition of a diploid cell given in Section VIII.

Dodge says: "only diploid nuclei can be heterozygous" so that in "hybrid" dikaryotic mycelia "pink could not be dominant over white." The facts concerning colour dominance in dikaryotic mycelia and fructifications as obtained by Zattler and by Johnson and Newton are indisputable and must be accepted. This being so, it can no longer be maintained that "only diploid nuclei can be heterozygous." It is true that a *single* haploid nucleus, such as that in a spermatozoon or in a cell of a haploid mycelium, can not cause dominance. It takes two genoms or haploid sets of chromosomes to produce dominance; but, so long as both sets are present in one cell, for the production of dominance it matters not whether they are in one diploid nucleus or in two haploid nuclei.

While very willingly one acknowledges that Dodge's beautiful experiments have proved that the phenomenon of dominance can be well displayed in an ascus of a Neurospora after the conjugate nuclei in the young ascus have fused to form a diploid nucleus, yet one cannot accept his view that, in the Higher Fungi in general, dominance can be displayed only in asci and basidia. The phenomenon of dominance may be shown not only in asci and basidia but also in the dikaryotic mycelia and dikaryotic fructifications of Basidiomycetes and in the dikaryotic ascogenous hyphae of Ascomycetes.

Dodge (1939) also objects to the use of the term *hybrid* for mycelia and fructifications of the Higher Fungi produced by crossing different races; but I consider the use of the term hybrid in this connexion fully justified.

Dodge says: "It is difficult to see how one could obtain a true

hybrid mycelium under normal conditions. The larger part of fruit bodies of mushrooms and ascomycetes is haploid; therefore we should not use the term hybrid to describe a mere intermingling of hyphae or nuclei of two different races to form the framework of such structures. If commercially valuable as mushrooms they could be propagated as spawn and given a trade name. They are a sort of chimaera or graft hybrid and any apparent dominance is a false one".

Accepting the view that I have supported with much evidence in previous Sections, namely, that a complete set of pairs of homologous chromosomes held collectively in two haploid nuclei of a conjugate pair affects the cytoplasm of the cell in the same way as does a complete set of pairs of homologous chromosomes held within a single diploid nucleus, it follows that we must admit that true hybrids can be formed by crossing differing races in the Higher Fungi.

Dodge states that "the larger part of the fruit-bodies of mushrooms and ascomycetes is haploid" and that in these structures we have "a mere intermingling of hyphae or nuclei of two different races" so that the fruit-bodies are "a sort of chimaera or graft hybrid". Here it must be pointed out that, in hybrid fructifications of Hymenomycetes, Rust Fungi, and other Basidiomycetes, it is not true that there is "a mere intermingling of hyphae or nuclei of different races". In such fructifications the rule is that all the cells contain two haploid nuclei of opposite sex and are therefore dikaryotic diploid cells. Thus there is no intermingling of monokaryotic haploid hyphae of different races in any hybrid basidiomycetous fructification.

Hybrid fructifications of Ascomycetes differ in structures from those of the Basidiomycetes, for they contain not only dikaryotic diploid cells, those of the ascogenous hyphae and very young asci, but also many haploid cells, those of the peridium, the fleshy part of the sporocarp, and the paraphyses in the hymenium. A protoperithecium of such a Pyrenomycete as *Neurospora sitophila* is formed on a haploid mycelium and only part of it—its oogonium—becomes diploidised, whereas in such a Hymenomycete as *Coprinus lagopus* a fruit-body is formed on a dikaryotic diploid mycelium and, therefore, from the first is composed entirely of dikaryotic diploid cells.

It thus appears that, in plants and animals in general, there are three kinds of hybrids produced by crossing that can be distinguished from one another: (1) those that are *synkaryotic,* (2) those that are *dikaryotic,* and (3) those that are *partly monokaryotic* and *partly dikaryotic.*

(1) A *synkaryotic hybrid* may be defined as a hybrid made up of synkaryotic cells, *i.e.* cells whose nuclei are diploid.

Examples: hybrid animals; hybrid Mosses, Ferns, and Flowering Plants; and the hybrid yeasts produced by Winge and Laustsen (1939, b).

(2) A *dikaryotic hybrid* may be defined as a hybrid made up of dikaryotic diploid cells, *i.e.* cells containing a conjugate pair of haploid nuclei.

Examples: hybrid mycelia and fructifications of Hymenomycetes, Gastromycetes, Uredinales, Ustilaginales, Septobasidiales, and other Basidiomycetes.

(3) A *monokaryotic-dikaryotic hybrid* may be defined as a hybrid made up in part of monokaryotic haploid cells and in part of dikaryotic diploid cells.

Examples: hybrid perithecia of Pyrenomycetes such as are obtained by crossing differing races of *Neurospora sitophila;* hybrid fructifications of Discomycetes and other Ascomycetes.

A fourth kind of hybrid may also be recognised, namely, a hybrid heterokaryotic mycelium and its perithecia produced by the mating of differing races derived from occasional unisexual dwarf-ascospores in such exceptional Pyrenomycetes as *Neurospora tetrasperma* (Shear and Dodge, 1927; Dodge, 1927), *Pleurage anserina* (Dowding, 1931; Ames, 1934; Dodge, 1936), and *Gelasinospora tetrasperma* (Dowding, 1933) in which, normally, the asci are 4-spored and each ascospore, when first formed, contains one (+) nucleus and one (−) nucleus. In the hybrid mycelia are nuclei of both races comingled in each cell but not, so far as is known, arranged in conjugate pairs. In the perithecia produced on the hybrid mycelium the asci contain four spores and each ascospore contains a (+) nucleus and a (−) nucleus. One of the nuclei of an ascospore may contain one factor of the pair in which the original parents differed and the other nucleus may contain the other factor. When such an ascospore germinates it gives rise to a hybrid mycelium from the very first, and this hybrid mycelium resembles in

principle the hybrid mycelium formed by mating the two unisexual mycelia of differing races derived from dwarf ascospores. This fourth kind of hybrid, to distinguish it from a dikaryotic hybrid of the Basidiomycetes, may be called a *heterokaryotic hybrid*. It will now be defined.

(4) A *heterokaryotic hybrid* may be defined as a hybrid made up of heterokaryotic cells, *i.e.* cells containing a mixture of (+) and (–) nuclei not arranged in conjugate pairs.

Examples: hybrid mycelia and perithecia of *Neurospora tetrasperma, Pleurage anserina,* and *Gelasinospora tetrasperma.*

Hybrids may also be classified according to their position in the life-history, with the zygote taken as the point of reference.

If we designate as *zygotes* those cells in which two nuclei of opposite sex fuse together, the zygote is: in Basidiomycetes, the probasidium or basidium; in Ascomycetes, the ascus; and in Mosses, Ferns, Flowering Plants, and animals, the fertilised egg-cell. On this basis we can divide hybrids into (1) *pre-zygotic,* and (2) *post-zygotic.*

(1) A *pre-zygotic hybrid* may be defined as a hybrid that is formed after the sexual process has been initiated by nuclear association but prior to the completion of the process by nuclear fusion.

Examples: the dikaryotic mycelial and fruit-body hybrids of Hymenomycetes, Gastromycetes, Uredinales, Ustilaginales, Septobasidiales, and other Basidiomycetes; the monokaryotic-dikaryotic hybrid fruit-bodies of Pyrenomycetes, Discomycetes, and other Ascomycetes.

(2) A *post-zygotic hybrid* may be defined as a hybrid that is formed after the sexual process has been completed by nuclear fusion.

Examples: the synkaryotic hybrids of Mosses, Ferns, Flowering Plants, and animals; and the synkaryotic hybrid Yeasts produced by Winge and Laustsen (1939, b).

X. THE DIKARYOTIC DIPLOID CELL IN PLANTS AND ANIMALS

In Mosses, Ferns, Flowering Plants, and animals in general, the only dikaryotic diploid cells are the egg-cells just after the entry of a male nucleus; whereas, in the Higher Fungi excluding the Yeasts, after the sexual process has been initiated, there is produced a long series of dikaryotic diploid cells terminating in the young basidia or young asci.

In Mosses, Ferns, and Flowering Plants the sporophyte, and in animals the animal body, is composed of synkaryotic diploid cells; whereas, in the Higher Fungi excluding the Yeasts, the only synkaryotic diploid cells are the basidia and the asci just after nuclear fusion has taken place.

In both animals and plants the formation of dikaryotic diploid cells is in all cases a step leading to nuclear fusion and the establishment of synkaryotic diploid cells.

In animals, Mosses, Ferns, and Flowering Plants, the dikaryotic diploid state of the ovum is *very transitory* and is quickly changed by nuclear fusion into the synkaryotic diploid state. In the Higher Fungi excluding the Yeasts, on the contrary, the dikaryotic diploid state is *long persistent;* for it is accompanied by conjugate nuclear division and the development of new cells, and it passes finally into the synkaryotic state only in the young basidia or young asci, *i.e.* in cells that are very distant in descent from the cells in which the first pairs of nuclei were originally established.

XI. HAPLOID, DIPLOID AND HETEROKARYOTIC MYCELIA

In the Basidiomycetes, the terms *haploid* and *diploid* can be applied not only to nuclei and to cells, but also to mycelia.

A *haploid mycelium* of a Hymenomycete or Rust Fungus is a mycelium whose *cells each contain a haploid nucleus.*

A *diploid mycelium* of a Hymenomycete or Rust Fungus is a mycelium whose cells *each contain a pair of conjugate nuclei.*

In such Pyrenomycetes as *Neurospora sitophila,* the ascospores are haploid and they give rise to a mycelium with several haploid nuclei in each cell. Here, too, we can speak of a *haploid mycelium.* However, in the Pyrenomycetes, a diploid mycelium strictly comparable with that of the Hymenomycetes and Rust Fungi is unknown. In the Pyrenomycetes, the only diploid cells containing pairs of conjugate nuclei are: the oogonium after the initiation of the sexual process, the cells of the ascogenous hyphae, and the young asci. These structures alone, in the Pyrenomycetes, are truly diploid.

In the Pyrenomycetes, the nearest approach to the diploid mycelium of the Hymenomycetes and Rust Fungi is the mycelium of such aberrant species as *Neurospora tetrasperma* (Dodge, 1927), *Pleurage anserina* (Dowding, 1931; Ames, 1934; Dodge, 1936),

and *Gelasinospora tetrasperma* (Dowding, 1933). Here, the asci contain four spores instead of the usual eight, and each of the four spores of an ascus encloses nuclei of both sexes. These bisexual ascospores, on germination, produce bisexual mycelia in each cell of which there is a mixture of nuclei of both sexes. These nuclei do not appear to be arranged in conjugate pairs. Thus a normal mycelium of *N. tetrasperma, P. anserina,* or *G. tetrasperma,* while not diploid in the sense that it possesses pairs of conjugate nuclei, yet resembles the strictly diploid mycelia of Hymenomycetes and Rust Fungi in that it is bisexual.

A haploid mycelium or a multicellular trichogyne of *Neurospora sitophila* or similar heterothallic Pyrenomycete, through which nuclei of opposite sex are passing *en route* to the ascogonium in a proto-perithecium, where they are destined to take part in the formation of the first pair or pairs of conjugate nuclei, may be described as *heterokaryotic,* but not as diploid (in the sense of containing pairs of conjugate nuclei).

Also, in *Neurospora tetrasperma, Pleurage anserina, Gelasinospora tetrasperma,* and similar homothallic Pyrenomycetes, the bisexual mycelium derived from a normal bisexual ascospore may be described as *heterokaryotic* or as *mictohaplontic* (Kniep, 1928, 1929), but not as diploid.

XII. HETEROKARYOTIC MYCELIA IN RUST FUNGI

In the Rust Fungi, *e.g. Puccinia graminis,* a haploid mycelium derived from a basidiospore may be invaded by one or more nuclei of opposite sex that have entered the mycelium *via* ordinary vegetative hyphae (as when two mycelia of opposite sex have fused together) or *via* flexuous hyphae (as when the nectar of (+) and (–) pustules has been mixed).

In such a Rust as *Puccinia graminis,* just as in the Pyrenomycete *Neurospora sitophila,* a haploid mycelium through which nuclei of opposite sex are passing to their destinations—in *P. graminis* the basal cells of the proto-aecidia—may be described as *heterokaryotic* but not as diploid, for in the mycelium no conjugate pairs of nuclei are formed.

In *Puccinia graminis,* the basal cells of the proto-aecidia are the first cells in which conjugate pairs of nuclei are established. They develop into aecidiosporophores that abstrict chains of dikaryotic

aecidiospores. The aecidiospores germinate on wheat-plants, etc., and give rise to mycelia whose cells are provided with conjugate nuclei. These mycelia give rise to dikaryotic uredospores and these, in turn, give rise to dikaryotic mycelia.

To sum up, for *Puccinia graminis* and similar heterothallic Rust Fungi it can be said that: (1) the mycelia derived from basidiospores are *at first haploid* and *then heterokaryotic;* and (2) the mycelia derived from aecidiospores and uredospores are *diploid.*

XIII. STAGES IN THE SEXUAL PROCESS OF PLANTS AND ANIMALS

In Mosses, Ferns, and Flowering Plants and in animals generally, the *sexual process* has two stages: (1) *nuclear association,* and (2) *nuclear fusion.*

In the Higher Fungi (Basidiomycetes and Ascomycetes) the *sexual process* has three stages: (1) *nuclear association,* (2) *conjugate nuclear divisions,* and (3) *nuclear fusion.*

XIV. NUCLEAR ASSOCIATION

By *nuclear association* is meant the coming together in a single cell of two nuclei of opposite sex. The phenomena leading to the accomplishment of nuclear association are varied and complicated: two gametes or haplonts must be brought into contact with one another and then, somehow or other, the male nucleus or its equivalent must be introduced into an egg or other cell of opposite sex.

In Mosses, Ferns, Flowering Plants, and animals generally, nuclear association is accomplished in a single cell, the *ovum;* but, in the Hymenomycetes and Rust Fungi, it is accomplished not in a single cell but in many cells of the mycelium (Hymenomycetes) or proto-aecidia (Rust Fungi) progressively, so that not one pair of nuclei of opposite sex but many such pairs come into existence. In *Coprinus lagopus,* after a large haploid mycelium (*AB*), 6 cm. wide, has been inoculated at its edge by a small 1 mm.-wide haploid mycelium (*ab*) of opposite sex, it takes about three days for the large mycelium to receive, in all the tens of thousands of cells that make up its growing hyphae, the tens of thousands of nuclei of opposite sex required to provide conjugate mates for all its own nuclei (Buller, 1930, 1931). Evidently, the few (*ab*) nuclei that migrate from the small (*ab*) mycelium into the large (*AB*) mycelium increase greatly in number by nuclear division *en route* to their destinations.

XV. THE DIPLOIDISATION PROCESS

Having noted in *Coprinus lagopus* the slow progress and complexity of nuclear association, I felt obliged to regard nuclear association in the Hymenomycetes as a process worthy of a special study and of a special name.

Therefore, in 1930, for the Hymenomycetes, I introduced the term *diploidisation* which I defined as *the process by which a haploid cell is converted into a diploid cell or a haploid mycelium into a diploid mycelium by the formation of a pair or pairs of conjugate nuclei within the cell or within the mycelium* (Buller, 1930, 1931).

In Hymenomycetes like *Coprinus lagopus* the diploidisation process is completed when a haploid mycelium has been converted into a dikaryotic diploid mycelium. During the transformation the haploid cells become dikaryotic diploid cells.

In 1930, also, I introduced the verb *to diploidise*. Thus, in the Hymenomycetes, a haploid cell or a diploid cell is said to *diploidise* a haploid cell when, through its agency, the latter becomes converted into a diploid cell containing conjugate nuclei, $(n) + (n)$; and a haploid or diploid mycelium is said to *diploidise* a haploid mycelium when, through its agency, the latter becomes converted into a diploid mycelium containing pairs of conjugate nuclei, $(n) + (n)$.

XVI. DISCUSSION OF TERMINOLOGY

The useful term *conjugate nuclei* was introduced by Poirault and Raciborski in 1895 in the course of their description of the aecidia and aecidiospores of the Uredinales, and they applied it to two nuclei that are associated with one another in one and the same cell and that divide simultaneously with the long axes of their spindles side by side and parallel to one another. In 1902, Maire referred to such a pair of nuclei in a young aecidium as a *synkaryon;* but, in 1912, he proposed that the pair of nuclei should be called a *dikaryon* and that the term *synkaryon* should be restricted to the fusion nucleus in a teleutospore.

In treating of the nuclear condition of the Hymenomycetes, Gastromycetes, Uredinales, Pyrenomycetes, Discomycetes, and other Higher Fungi, instead of the set of terms usually employed by myself, an alternative set of terms is permissible. The two sets of terms may be listed side by side as follows.

	Terms employed by the writer.	*An alternative set of terms.*
(1)	Haploid cell	Monokaryotic cell
(2)	Haploid mycelium	Monokaryotic mycelium
(3)	Haploid fruit-body	Monokaryotic fruit-body
(4)	Conjugate nuclei	Dikaryon
(5)	Conjugate nuclear division	Dikaryotic nuclear division
(6)	Diploid cell	Dikaryotic cell
(7)	Diploid mycelium	Dikaryotic mycelium
(8)	Diploid fruit-body	Dikaryotic fruit-body
(9)	Diploidise	Dikaryotise
(10)	Diploidisation	Dikaryotisation
(11)	Haplophase	Monokaryophase
(12)	Diplophase	Dikaryophase.

So far as I know, the terms *dikaryotic nuclear division* and *dikaryotise* are of my own invention, but the term *dikaryotisation* is in use and has been employed by Linder (1940).

I prefer the first of the two sets of terms to the second because: (1) the terms of the first set are somewhat less polysyllabic and are more euphonious than those of the second set; (2) the adjectives *haploid* and *diploid* indicate the relative number and the nature of the chromosomes in the cells of the mycelia or fruit-bodies, whereas the corresponding adjectives *monokaryotic* and *dikaryotic* seem to indicate merely the number of nuclei in the cells; and (3) the term *conjugate nuclei* indicates that the nuclei are yoked together and undergo conjugate division, whereas the term *dikaryon,* while indicating that there are two nuclei in a cell, is less suggestive of the fact that the nuclei divide conjugately.

The terms *diploidise* and *diploidisation* have already been adopted: for the Hymenomycetes, by Quintanilha (1937–1939), Brodie (1939), Oikawa (1939), and others; and, for the Uredinales, by Brown (1932), Lamb (1935), Oliveira (1940), and others.

Brodie (1936), in treating of the formation of oidia in Hymenomycetes, followed the suggestion of Bessey (1935) and employed the terms *monocaryon mycelium* and *dicaryon mycelium* instead of *haploid mycelium* and *diploid mycelium* respectively because, as he said, "we do not as yet know whether or not the influence exercised by the two nuclei of a dicaryon upon the cell which contains them is the same as that of a single diploid nucleus." The evidence brought forward in Sections VI and VII of this review proves conclusively that the two nuclei of a conjugate pair do actually affect the cytoplasm in the same way as a diploid nucleus. Brodie's ob-

jection to the use of the terms *haploid mycelium* and *diploid mycelium* thus falls to the ground.

Since *karyokinesis* has always been spelled with a *k* and not with a *c*, I prefer to spell such words as *monokaryon* and *dikaryon* with a *k* rather than with a *c*. The employment of the nouns *monokaryon* and *dikaryon* as adjectives, when applied to the word mycelium, results in the formulation of harsh combinations. I therefore prefer *monokaryotic mycelium* to *monokaryon mycelium* and *dikaryotic mycelium* to *dikaryon mycelium*.

Brodie (1936) admitted that, since he used the terms *monocaryon mycelium* and *dicaryon mycelium,* he was inconsistent in using the term *diploidisation;* but he added: "the writer prefers, however, not to suggest a substitute for this most convenient term." The substitute in the alternative set of terms is the seven-syllable word *dikaryotisation;* but how much neater and more significant seems such a sentence as "Two *haploid mycelia* of opposite sex *diploidise* one another and convert one another into *diploid mycelia*" than the equivalent sentence "Two *monokaryon mycelia* of opposite sex *dikaryotise* one another and convert one another into *dikaryon mycelia*."

The terms *primary mycelium* and *secondary mycelium* (Falck, 1909) have been used in studies of the Hymenomycetes by Bensaude (1918) and Quintanilha (1937–1939). I prefer their equivalents, *haploid mycelium* and *diploid mycelium,* because these terms are briefer and indicate not mere succession but chromosomal content of the mycelial cells.

The terms *haploid mycelium* and *diploid mycelium* have been reduced in form by Kniep (1928) to *haplomycelium* and *diplomycelium* respectively. To this no serious objection can be made; but if such one-word terms as *haplocell, diplocell, haplofruitbody,* and *diplofruitbody* had been invented, I should avoid them on account of their hybrid origin.

The sexual process in the Basidiomycetes and certain Ascomycetes is referred to by Gäumann and C. W. Dodge (1928) as *pseudomixis* and they state that this is equivalent to Hartmann's *pseudogamy* and signifies a sexual process in which the copulating cells are not gametes but vegetative cells of the thallus. The terms pseudomixis and pseudogamy both suggest that the eventual union of the nuclei is illusory and does not represent a true sexual proc-

ess. As a matter of fact, in each basidium or ascus there takes place a union of two nuclei, one derived from one parent and the other from the other parent, followed by meiosis, the sexual nature of which cannot be denied; and the sexual process of such fungi as *Coprinus lagopus* and *Neurospora sitophila* is just as perfect as that of the Fruit-fly, *Drosophila melanogaster,* or the Easter Lily, *Lilium longiflorum.* Therefore, in describing the sexual process of the Higher Fungi, the terms pseudomixis and pseudogamy should be avoided. Kniep (1928) has already rejected them in so far as they have been applied to the Discomycetes, and in their place he has adopted Renner's (1916) much better term *somatogamy.*

The haploid mycelia of the Higher Fungi are *unisexual.* In describing genetic experiments made with such mycelia, it is convenient, with Kniep (1928), Zattler (1924), and others, to refer to a haploid mycelium as a *haplont* (haploid soma) and to a dikaryotic mycelium or fruit-body produced as a result of the mating of two haploid mycelia as a *diplont* (diploid soma).

In breeding experiments with such a fungus as *Coprinus lagopus,* when a pair of non-sexual Mendelian factors is involved, say D and d where D is dominant and d recessive, three types of dikaryotic diploid mycelia are possible. Since these mycelia contain conjugate pairs of haploid nuclei and not diploid nuclei, they should be represented not by the symbols (DD), (Dd), and (dd), but by the symbols $(D)+(D)$, $(D)+(d)$, and $(d)+(d)$. One may use current genetic terms applied to the sporophytes of Higher Plants and the bodies of animals and describe: the mycelium $(D)+(D)$ as *homozygous* and as a *pure dominant;* the mycelium $(D)+(d)$ as *heterozygous* and as an *impure dominant;* and the mycelium $(d)+(d)$ as *homozygous* and *recessive.* However, none of these mycelia should be referred to as a zygote; for the basidium, in which two nuclei of opposite sex fuse together, alone deserves this name.

Gäumann and C. W. Dodge (1928) have referred to the dikaryotic mycelium of Basidiomycetes as a *binucleate zygote* and to the basidia in which nuclear fusion takes place as *zeugites.* This terminology must be rejected. On the basis of comparative morphology, it seems more logical to conceive of the basidia in which the sexual process is completed by nuclear fusion as zygotes and to

regard the dikaryotic mycelium as a pre-zygotic structure. If this be granted, the term zeugite for a basidium can be discarded. Similarly, in Ascomycetes, the asci are to be regarded as zygotes and the ascogenous hyphae as the elements of a pre-zygotic structure. A revised definition of a zygote that includes basidia and asci along with fertilised egg-cells will be given in Section XVIII.

When two gametes meet and fuse and form a zygote, the first step in the process has been called *plasmogamy* (a union of two cytoplasms) and the second step *karyogamy* (nuclear fusion). These terms have also been employed in describing the sexual process of the Higher Fungi (Gäumann and Dodge, 1928). From the point of view of inheritance, the chief significance of the first step in the sexual process is not the union or mixing of two masses of cytoplasm but rather the bringing of two nuclei of opposite sex into intimate association in one and the same cell. Therefore, in Section XIII, in listing the stages of the sexual process in plants and animals in general I have designated the first stage not as plasmogamy, but as *nuclear association.* As long ago as 1904, this term was used by Blackman in describing the sexual process of the Uredinales.

In the Higher Fungi, for reasons already given in Section XV, I have called the first step in the sexual process *diploidisation.* During diploidisation in such a fungus as *Coprinus lagopus* or *Neurospora sitophila,* when the process is initiated by the fusion of two mycelia of opposite sex, there is contact and union of two cytoplasms only in the common tubes formed by the hyphal anastomoses, whereas there is much travelling of nuclei of one sex through the cytoplasm of the hyphae of opposite sex in search of conjugate partners. On this account, the first step in the sexual process of the Higher Fungi is better described as diploidisation than as plasmogamy.

In the Higher Fungi, *e.g. Coprinus lagopus, Ascobolus magnificus,* and *Pleurage anserina,* hyphal fusions between adjacent hyphae of a single haploid mycelium or between the hyphae of two haploid mycelia of one and the same sex are very frequently formed (Buller, 1933). In the common tube of every fusion two different masses of cytoplasm become continuous with one another. Here we have plasmogamy that is entirely non-sexual.

Finally, a word may be said concerning the terms employed for

certain haploid rudiments of fructifications in the Uredinales and Pyrenomycetes.

The fundaments of the aecidia (aecia) or 'rudimentary aecidia', which in such Rusts as *Puccinia graminis* and *P. helianthi* become diploidised and then develop into aecidia, require a special name. Therefore, in 1938, in a letter to *Nature,* I proposed that they should be called *proto-aecidia.* Already (1940, a) Johnson and Newton have adopted the shortened form of this term, namely *proto-aecia,* in describing a photomicrograph of a normal haploid mycelium of *P. graminis.*

In Rusts like *Puccinia suaveolens* in which the aecidial stage has been dropped from the life-cycle, the haploid rudimentary structure that gives rise to a uredospore pustule or uredium may be called a *proto-uredospore pustule* or *proto-uredium.*

In the spring of 1939, at the New York Botanical Garden, while discussing with Dr. B. O. Dodge the life-history and genetics of *Neurospora sitophila,* I proposed that the rudimentary haploid perithecia that develop so freely on the haploid mycelia of *N. sitophila* and similar Pyrenomycetes and that had previously been referred to by other investigators as *sclerotia, sclerotial bodies, bulbils,* and *perithecial fundaments* should henceforth be called *proto-perithecia;* and this suggestion met with his instant approval.

The great advantage of the use of such terms as *proto-aecidium* or *proto-aecium, proto-uredium,* and *proto-perithecium* is that they are devoid of any suggestion of female sexuality and merely indicate that the haploid structures involved are destined, after diploidisation has been effected, to develop into certain fructifications of which they are the fore-runners.

In Hymenomycetes like *Coprinus lagopus,* every growing haploid hypha is capable of being diploidised and is therefore what we may call a *pro-diploidisation hypha.* In a haploid mycelium of a Rust, such as *Puccinia graminis,* the pro-diploidisation hyphae make up the so-called fertile cells at the base of each proto-aecidium; while, in Pyrenomycetes and Discomycetes, the pro-diploidisation hyphae are the ascogonia of the ascocarps. In these ascogonia there is often a reduction of the diploidisation possibility to a single *pro-diploidisation cell.* This we find in such a Discomycete as *Ascobolus magnificus* (Dodge, 1920). Such a cell is certainly not an egg-cell, for the sexual process is only begun there and its consummation is accomplished in the asci.

Linder (1940) holds that "the Uredinales or an ancestral form of this order should be considered to be the primitive Basidiomycetes," and he supports his contention with morphological considerations drawn chiefly from a comparison of basidia. With this view I cannot agree. It seems to me most unlikely that the Uredinales, provided with haustoria and so highly specialised in relation to their host-plants that no one has ever succeeded in cultivating them saprophytically, should have given rise to the whole of the great saprophytic series of fungi represented by the Hymenomycetes and their allies. Just as in the animal kingdom one can easily imagine how parasitic animals have been derived from non-parasitic, but not the reverse, so in the plant kingdom one can find many cases where one seems obliged to conclude that parasitic plants have been derived from non-parasitic and not non-parasitic plants from parasitic. Thus, in the Convolvulaceae, we have Evolvulus, Convolvulus, and Cuscuta. Surely here, the trend of evolution has been from the non-climbing genus, through the climbing genus, to the climbing parasitic genus. On the basis of considerations just suggested, I am inclined to favour the speculation that, in the course of evolution, the lower Hymenomycetes (Thelephoraceae) and their allies, as saprophytes, threw off various groups of parasites of which the Uredinales, the Ustilaginales, and the Exobasidiales are examples.

If the Uredinales sprang from one of the lower Hymenomycetes, then we must suppose that the pro-diploidisation hyphae, which in the parental Hymenomycete were all the growing hyphae of the mycelium, came to be restricted in the Uredinales, as this group became more and more specialised for parasitising the green leaves of its host-plants, to the basal (fertile) cells of the proto-aecidia.

XVII. THE IDENTIFICATION OF GAMETOPHYTIC AND SPOROPHYTIC STAGES IN THE LIFE-HISTORIES OF THE HIGHER FUNGI

The terms *gametophyte* and *sporophyte* were invented as names for the alternate generations in Mosses, Ferns, and Flowering Plants. Subsequently, they were applied to certain stages in the life-histories of the Higher Fungi.

In the past, various writers on the Basidiomycetes have regarded a *haploid mycelium* as an *n generation* or *gametophyte,* and a *diploid mycelium and fructification* as a *2n generation* or *sporophyte.*

For the Uredinales, such an alternation of generations, *i.e.* of gametophytes and sporophytes, is clearly set forth in a life-history diagram by Grove (1913); and a similar diagram containing the terms "*gametophyte* or *haplophase*" and "*sporophyte* or *diplophase*" was published by Arthur in 1929.

Also Hartmann (1929), in his discussion of sex in the Hymenomycetes, has illustrated his conception of the life-history of an agaric by means of a diagram in which are two divisions, the first labelled "diplophase = sporophyte" and the second "haplophase = gametophyte."

In a Moss or Fern, the gametophyte forms unicellular gametes, a spermatozoid fertilises an ovum, and the zygote thus produced gives rise to a sporophyte. There is nothing strictly comparable with this in the Higher Fungi. Thus, in the Hymenomycete *Coprinus lagopus,* when two multicellular haploid mycelia of opposite sex meet and fuse, they diploidise one another's vegetative cells and the production of zygotes is delayed until the formation of the basidia. If the two haploid mycelia in a successful combination are to be regarded as gametophytes or as multicellular gametes, so also must they be after they have been diploidised; for, as I discovered (*vide infra*), a diploid mycelium containing pairs of conjugate nuclei can diploidise haploid mycelia with which it comes into contact. Functionally, both the haploid and the diploid mycelia of such a fungus as *Coprinus lagopus* are gametophytes or multicellular gametes. As Brown found (1932), the same is true for a Rust like *Puccinia helianthi.*

Also, the fruit-bodies of *Coprinus lagopus* are diploid and contain pairs of conjugate nuclei, just like the mycelia on which they are borne. From pieces of the fruit-body, *e.g.* hyphae of the stipe, vegetative mycelia can be grown, and these diploid mycelia can be used to diploidise haploid mycelia derived from basidiospores. Therefore, potentially, even a fruit-body (sporophore) has gametophytic properties.

It is true that the diploid mycelium and the diploid fruit-bodies of *Coprinus lagopus* eventually give rise to basidiospores and to that extent are sporophytic; but they differ from the diploid sporophyte of a Moss or a Fern in that they have gametophytic potentialities. The sporophyte of a Fern cannot fertilise an ovum in an archegonium of the prothallial gametophyte, but the mycelial part

of the so-called sporophyte of a *Coprinus lagopus* can, and no doubt under natural conditions often does, diploidise a haploid mycelium.

A diploid mycelium and diploid fruit-body of *Coprinus lagopus* and other Basidiomycetes should be represented by the symbol $(n) + (n)$ and not by the symbol $(2n)$. Those who accept the Grovian scheme for the life-histories of Hymenomycetes and Rust Fungi have not perceived that, so far as nuclear activity and physiological potentialities are concerned, the equation

$$(2n) = (n) + (n)$$

in which n is the number of chromosomes and a pair of brackets represents a nucleus, is not exactly true.

While a vascular Fern plant is a sporophyte and nothing more, a diploid mycelium of *Coprinus lagopus,* formerly regarded as part of a sporophyte, as we have seen is now known to have gametophytic properties. Such a diploid mycelium therefore seems to be both sporophytic and gametophytic; but, where there is an alternation of generations, one cannot admit that a single structure belongs to both generations! It thus appears that the old conception of a diploid mycelium as part of a sporophyte was not well based.

If a parallel between the life-histories of a Fern and a Hymenomycete, involving an alternation of generations, must still be sought, then, as will now be shown, a diploid mycelium of such a fungus as *Coprinus lagopus* should be regarded not as a stage of the sporophyte, but as a stage of the gametophyte.

In a Moss, Fern, or Flowering Plant, the gametophytic generation comes to an end with the fertilisation of an egg-cell and the production of a zygote, and it is the zygote that is the first cell of the sporophyte. In *Coprinus lagopus* and similar fungi, the basidia are the zygotes, for in them alone nuclear fusion takes place. If the *production of a zygote* marks the end of the gametophytic generation and the beginning of the sporophytic generation in a Fern, so should it do in *Coprinus lagopus*. If this is admitted, then in *C. lagopus* the *gametophytic generation* includes basidiospore, haploid mycelium, diploid mycelium, fruit-body, and young basidia, while the *sporophytic generation* includes merely the young basidium just after nuclear fusion and the basidium during meiosis and the production of the four haploid basidiospores. Such a division of the life-history of *Coprinus lagopus* into a very long gameto-

phytic phase and an exceedingly brief sporophytic phase, based as it is on the place of production of zygotes is consonant with the fact that, functionally, the haploid mycelium, the diploid mycelium, and the fruit-bodies all have gametophytic potentialities.

The new conception of the parallelism between the life-history of a Hymenomycete and that of a Fern, as outlined above, involves the reduction of the sporophyte in the Hymenomycetes to a basidium in the intermediate and later stages of its development. Perhaps this *reductio ad absurdum* will satisfy those who delight in comparing the life-histories of unrelated plants. For my own part, in treating of sex in the Higher Fungi, I have always avoided (and I still intend to avoid) using the terms gametophyte and sporophyte.

In the Uredinales, *e.g. Puccinia graminis,* on the basis of what has just been said: the *gametophytic generation* includes basidiospore, haploid mycelium derived from a basidiospore, pycnidia and pycnidiospores, proto-aecidia, diploidised proto-aecidia, aecidia, aecidiospores, mycelium derived from an aecidiospore, uredospores, mycelium derived from a uredospore, and the young binucleate cells of the teleutospores; while the *sporophytic generation* is limited to the cells of the teleutospores after nuclear fusion has taken place (zygotes), and to the basidium during meiosis and the production of the four haploid basidiospores.

In Pyrenomycetes like *Neurospora sitophila,* again on the basis of the discussion of the life-history of *Coprinus lagopus,* the *gametophytic generation* includes ascospore, haploid mycelium derived from an ascospore, macroconidiophores and macroconidia, microconidiophores and microconidia, proto-perithecia with their envelope, oogonia, and trichogynes, the oogonia after they have been diploidised, the ascogenous cells, and the young binucleate asci; while the *sporophytic generation* is limited to the asci after nuclear fusion has taken place (zygotes) and during meiosis and the formation of the eight haploid ascospores.

As indicated above, the sporophyte in Hymenomycetes, Uredinales, and Pyrenomycetes is limited to a basidium, a teleutospore with basidia, and an ascus respectively. Since these structures are not distinct or independent plants, they cannot be regarded as really homologous with the sporophytes of Mosses, Ferns, and Flowering Plants.

In discussing the life-history of the Hymenomycetes and Rust Fungi some writers have used the terms *monokaryophyte* and *dikaryophyte* to indicate a fungus in its *haploid* stage and its *diploid* stage respectively. The term *dikaryophyte* applies to a diploid mycelium and the diploid fruit-bodies that it produces, so that a diploid mycelium is merely the vegetative stage of a dikaryophyte. In using the terms under discussion it must be remembered that a monokaryophyte does not produce a dikaryophyte but, on being diploidised, becomes *converted* into a dikaryophyte. I feel that the terms monokaryophyte and dikaryophyte are somewhat suggestive of the terms gametophyte and sporophyte and, partly for this reason, I have hitherto avoided using them.

In the Higher Fungi, as has now become apparent, the "(n) generation" of Grove and others is not the whole of the "gametophyte", and the "$(2n)$ generation" ($= (n) + (n)$ generation) of the same authors is not the "sporophyte". In treating of the life-histories of Basidiomycetes and Ascomycetes, we are not justified in using expressions like *(n) generation, $(2n)$ generation, gametophyte,* and *sporophyte* that imply the existence of an alternation of generations comparable with that in Mosses, Ferns, and Flowering Plants; and we should be content merely with dividing a life-history into a *haplophase* and a *diplophase* or, if one wishes, into a *monokaryophase* and a *dikaryophase*.

In the life-history of plants and animals that have sexual reproduction we may recognise, if we wish, not only a haplophase and a diplophase but also a *zygophase* and a *meiophase*.

A *zygophase* may be defined as the phase of the life-history during which the zygote is formed and continues to exist until it begins to divide. In Mosses, Ferns, Flowering Plants, and animals, the zygote gives rise to a diplont, whereas in the Higher Fungi it gives rise to haploid basidiospores or ascospores.

A *meiophase* may be defined as the phase of the life-history during which a diploid nucleus undergoes reduction so that it gives rise to haploid nuclei, usually four in number. These haploid nuclei become enclosed in gametes or haploid cells.

If we recognise a zygophase and a meiophase as phases in the life-history, then in the life-history of Mosses, Ferns, Flowering Plants, and animals the sequence of phases is:

haplophase ⟶ zygophase ⟶ diplophase ⟶ meiophase

while, in the life-history of Basidiomycetes and Ascomycetes, the sequence of phases is:

haplophase ⟶ diplophase ⟶ zygophase ⟶ meiophase

XVIII. FERTILISATION, CONJUGATION, AND THE ZYGOTE

Such terms as *fertilisation* and *conjugation,* used so successfully in connexion with the sexual processes of Algae, Lower Fungi (Phycomycetes), Ferns, Mosses, Flowering Plants, and animals, cannot be employed in connexion with the sexual process of the Hymenomycetes owing to the special organisation of these fungi. An egg is said to be *fertilised* as soon as the male nucleus has fused with the female nucleus; but when, in *Coprinus lagopus,* two haploid mycelia of opposite sex have been mated, the immediate result is the formation in the mycelia of many pairs of conjugate nuclei; and there is no nuclear fusion until fruit-bodies have been formed and young basidia have come into existence. The young basidia are not egg-cells and, from the first, they contain a pair of conjugate nuclei. One cannot therefore speak of them as having been fertilised. Since in each young basidium the two nuclei of the conjugate pair fuse together, we can, if we wish, call a basidium a *zygote.* An ascus, just after the fusion of its conjugate nuclei, may also be called a zygote.

The term *zygote* may here be re-defined. Formerly a zygote was defined as *the product of the fusion of two gametes.* The underlying assumption was that after the union of the gametes the two nuclei soon fused together. To bring basidia and asci within the scope of the definition of a zygote, a *zygote* may be defined as *a cell in which two nuclei of opposite sex have fused together.*

Examples of the zygote as newly defined: fertilised egg-cells of animals and plants; the product of the union of isogametes of various Protozoa, Ulothrix, Spirogyra, and Mucoraceae; basidia of Hymenomycetes and Gastromycetes; cells of teleutospores (probasidia) in Rust Fungi; and asci of Ascomycetes.

XIX. DIPLOIDISATION IN RUST FUNGI AND PYRENOMYCETES

A *diploidisation process,* comparable with that in Hymenomycetes, takes place in Rust Fungi and Ascomycetes (Pyrenomycetes, Discomycetes) but, as might be expected, with some difference in detail.

As we have seen, in the Hymenomycetes, *e.g. Coprinus lagopus,* haploid mycelia of opposite sex *diploidise* one another, so that the cells of all the growing vegetative hyphae come to contain a pair of conjugate nuclei. As we have also seen, in the Rust Fungi, *e.g. Puccinia graminis,* and the Pyrenomycetes, *e.g. Neurospora sitophila,* the nuclei of one sex that have entered a haploid mycelium of opposite sex merely pass through the mycelium on their way to their destinations (proto-aecidia, proto-perithecia) and, whilst in passage, do not associate themselves conjugately with the nuclei that have been present in the mycelium from the first. Therefore, the haploid mycelia of opposite sex in Rust Fungi and Pyrenomycetes can be said to *heterokaryotise* one another but not to diploidise one another.

After the mating of two haploid mycelia of opposite sex, the first pairs of conjugate nuclei are established: in the Rust Fungi, in the basal cells of the proto-aecidia; and, in the Pyrenomycetes, in the oogonia of the proto-perithecia. We can therefore say that two mycelia of opposite sex *diploidise:* in the Rust Fungi, *the basal cells of one another's proto-aecidia;* and, in the Pyrenomycetes, *the oogonia of one another's proto-perithecia.*

Employing terms introduced above, one may describe the sexual process of *Neurospora sitophila* and similar Pyrenomycetes as follows. Two haploid mycelia (haplonts) of opposite sex heterokaryotise one another's vegetative hyphae and diploidise the ascogonia of one another's proto-perithecia. When the diploidisation process is complete, the ascogonia contain one or more pairs of conjugate nuclei. The diploidised ascogonia give rise to dikaryotic diploid ascogenous hyphae, and these hyphae eventually give rise to dikaryotic diploid asci. The conjugate nuclei in each young ascus soon undergo fusion, and thus the asci become converted into zygotes.

XX. DIPLOIDISATION IN PLANTS AND ANIMALS IN GENERAL

As a step toward generalising concerning the sexual process in plants and animals it is important to observe that the term *diploidisation process* and associated terms are applicable to the first part of the sexual process not only in the Higher Fungi (Basidiomycetes and Ascomycetes), but also in Mosses, Ferns, Flowering Plants, and the Animal Kingdom in general.

An ovum (egg-cell), just after a male nucleus has entered, is a dikaryotic diploid cell. Nuclear association has been accomplished. Here the *diploidisation process* has resulted in the establishment in the ovum of two nuclei of opposite sex. These two nuclei correspond to each of the numerous pairs of conjugate nuclei in a diploidised mycelium of such a Hymenomycete as *Coprinus lagopus.* We are therefore justified in saying that an ovum that has just passed from the uninucleate haploid stage to the dikaryotic diploid stage *has been diploidised.* Also from the point of view of the male gamete, in respect to such a dikaryotic ovum, we can say: for animals, a *spermatozoon has diploidised the ovum;* for Mosses and Ferns, a *spermatozoid has diploidised the ovum;* and, for Flowering Plants, a *male nucleus has diploidised the ovum.*

In the ova of Mosses, Ferns, Flowering Plants, and animals generally, the first stage of the sexual process—*diploidisation*—is soon accomplished and very transitory. The second stage of the sexual process—*nuclear fusion*—quickly sets in, and then the sexual process has been completed.

We thus perceive that a diploidisation process is the first stage of the sexual process in plants and animals in general.

XXI. DE-DIPLOIDISATION

In Basidiomycetes, while normally the two nuclei of a conjugate pair attract one another and divide simultaneously, thus providing pairs of conjugate nuclei often for thousands or even millions of new cells; yet, under certain conditions in some species, the two nuclei of a conjugate pair may become separated in different cells and these cells may develop into haploid hyphae. Thus a diploid mycelium may produce haploid cells or haploid branches. Here we have to do with a process which is just the reverse of diploidisation and which may therefore be called *de-diploidisation.* In the Ascomycetes, also, one case of de-diploidisation has been observed.

De-diploidisation may be thus defined: *in Basidiomycetes and Ascomycetes, the production of haploid cells or hyphae by a dikaryotic diploid mycelium or by a dikaryotic diploid cell.*

To illustrate the phenomenon of de-diploidisation in the Basidiomycetes seven special cases will now be described. Two are concerned with the production of haploid oidia by the mycelia of Hymenomycetes, two with the production of haploid proto-aecidia

(proto-aecia) and pycnidia (pycnia) by the mycelia of Uredinales, one with the production of haploid basidiospores by the basidia of certain Uredinales, and two with the production of haploid sporidia by the mycelia of Ustilaginales.

(1) In an agaric, *Pholiota aurivella,* it was observed by Vandendries and Martens (1932) that: a dikaryotic mycelium produces dikaryotic oidiophores, and that these give rise either to dikaryotic diploid oidia or to monokaryotic haploid oidia. In the latter case: a pair of conjugate nuclei (a dikaryon) enters a fusiform oidium; a cross-wall (septum) is then formed in the oidium, dividing it into two uninucleate cells; and, finally, the uninucleate cells break apart and become haploid oidia. The oidia on germination give rise to haploid mycelia.

(2) In another agaric, *Collybia velutipes,* it was observed by Brodie (1934) that: a dikaryotic mycelium gives rise not only to dikaryotic oidiophores and oidia, but also to uninucleate oidiophores and oidia; the uninucleate oidiophores and oidia are borne on uninucleate branches of the dikaryotic mycelium; the origin of the uninucleate branches is due to the separation of the two nuclei of a conjugate pair; and half of the oidia (those borne on certain haploid branches) are of one sex and the other half (borne on other branches) of opposite sex. The oidia on germination give rise to haploid mycelia that can be used to diploidise haploid mycelia of opposite sex.

(3) *Puccinia suaveolens* has a systemic mycelium that perennates in the roots of the Creeping Thistle, *Cirsium arvense.* This mycelium is dikaryotic and it arises from dikaryotic uredospores sown on Thistle seedlings (Buller and Brown, 1941). In the spring, the dikaryotic mycelium grows up into the new host-shoots and, on entering the rudiments of the developing leaves, becomes partially de-diploidised in that it there gives rise to haploid branches. These haploid branches produce haploid organs, namely, pycnidia (pycnia) and proto-aecidia (proto-aecia). Certain of the proto-aecidia on one thistle were caused to develop into diploid aecidia by applying to them the pycnidiospore-bearing nectar obtained from another thistle.

(4) A de-diploidisation of a diploid mycelium, similar to that just described, also takes place in *Puccinia minussensis* (Brown, 1941). The systemic mycelium of this Rust perennates in the

rhizomes of the Blue Lettuce, *Lactuca pulchella*. In the spring the diploid mycelium, (known to arise from diploid uredospores) grows up into the new host-shoots and sends haploid branches into the leaves. These branches produce haploid pycnidia and haploid proto-aecidia. Later, the proto-aecidia develop into diploid aecidia.

It appears that, in Rust Fungi with systemic mycelia that perennate in the subterranean organs of herbaceous perennial hosts, de-diploidisation of the diploid mycelium takes place annually and is an important phenomenon.

(5) In certain exceptional Rusts, *Endophyllum euphorbiae-sylvaticae* (Sappin-Trouffy, 1896; F. and Mme F. Moreau, 1918, 1919) and *Caeoma nitens* (Dodge and Gaiser, 1926), in connexion with the formation of the basidium there is no nuclear fusion whatsoever. In both these species: the basidium develops directly from an aecidiospore; the two nuclei of the conjugate pair in the aecidispore migrate into the young basidium; there they both divide once, thus giving rise to four haploid nuclei; and then the four nuclei pass through the four sterigmata into the four basidiospores. Thus the nuclei for the basidiospores are provided not by nuclear fusion followed by meiosis, but by the de-diploidisation of an unfused pair of conjugate nuclei.

(6) Also in the Ustilaginales (Smut Fungi), another group of Basidiomycetes, de-diploidisation may take place, and evidence of this fact is to be found in the observations of Bauch (1923) on *Ustilago longissima*, of Dickinson (1927) on *U. hordei* and *U. levis*, and of Hanna (1929) on *Ustilago zeae*. These authors obtained haploid sporidia from diploid mycelia.

(7) The morphology of *Tilletia tritici* and the behaviour of its nuclei have been discussed by myself and Vanterpool (Buller, 1933). As Boss (1927) observed, in *T. tritici*, after the conjugation of the highly modified stick-like sterigmata (Brefeld's *primary conidia*), two nuclei of opposite sex enter the primary basidiospore (Brefeld's *secondary conidium*); but, when this germinates and produces a mycelium, the two nuclei separate from one another and divide independently. The mycelium gives rise to secondary basidiospores (Brefeld's *secondary conidia*). Each of these receives but a single nucleus and is therefore haploid and unisexual. As Hanna found (Buller, 1933), in infection experiments, wheat plants inoculated with bisexual primary basidiospores or with a mixture of (+) and

(–) secondary basidiospores develop bunted heads, whereas wheat plants inoculated with either (+) or (–) secondary basidiospores do not develop bunted heads. Thus we have evidence that, in Smut Fungi, the phenomenon of de-diploidisation occurs not only in the Ustilaginaceae but also in the Tilletiaceae.

Finally, we may record a case of de-diploidisation that has been observed in association with an abnormal mode of development in *Saccharomyces ellipsoideus,* one of the Ascomycetes.

(8) As a rule, when two ascospores of a Saccharomyces fuse together, the two nuclei immediately unite and form a diploid nucleus. Renaud (1938), as recorded by Guilliermond (1940), observed in a wine yeast of the *Saccharomyces ellipsoideus* type an exceptional behaviour: in a zygote the two parental nuclei, instead of fusing, constituted a dikaryon which underwent conjugate nuclear division; the zygote budded off two or three concatenated dikaryotic cells; and karyogamy took place in the second or third cell. Renaud (1937) transferred a newly-formed zygote with as yet unfused nuclei to a moist chamber. The zygote then budded profusely, and some of the daughter cells received only one nucleus. Thus the two nuclei of certain pairs of conjugate nuclei had become separated from one another. In other words, de-diploidisation had taken place. The resulting haploid cells soon conjugated to form new zygotes.

XXII. THE BULLER PHENOMENON

Normally, in Hymenomycetes like *Coprinus lagopus,* when *two haploid mycelia* of opposite sex meet, they form hyphal fusions with one another, exchange nuclei, and diploidise one another.

Also, normally, in Hymenomycetes like *Coprinus lagopus,* when a *diploid mycelium* meets a *haploid mycelium,* the two mycelia form hyphal fusions with one another, and then *the diploid mycelium diploidises the haploid mycelium.*

The diploidisation of haploid mycelia by diploid mycelia has been called by Quintanilha (1937–1939) the *Buller phenomenon.* This phenomenon will now be discussed.

In *Coprinus lagopus* there are four kinds of basidiospores, (*AB*), (*ab*), (*Ab*), and (*aB*), that give rise to haploid mycelia with differing diploidising possibilities. Mutual diploidisation is effected in the two mycelia of the combinations (*AB*) × (*ab*) and (*Ab*)

$\times (aB)$ in each of which there are no factors in common, but not in the two mycelia of the combinations $(AB) \times (Ab)$, $(AB) \times (aB)$, $(ab) \times (Ab)$, and $(ab) \times (aB)$ in each of which there is one factor in common.

Diagrams illustrating steps in the diploidisation process of the combination $(AB) \times (ab)$ are given in my *Researches on Fungi* (1931).

In *Coprinus lagopus,* the combination $(AB) \times (ab)$ produces the dikaryotic diploid mycelium $(AB) + (ab)$, and the combination $(Ab) \times (aB)$ the dikaryotic diploid mycelium $(Ab) + (aB)$. Thus, in *Coprinus lagopus,* there are two kinds of dikaryotic mycelia.

I discovered (1930, 1931) that, in *Coprinus lagopus,* the diploid mycelia $(AB) + (ab)$ and $(Ab) + (aB)$ are able to diploidise haploid mycelia. It was shown that the diploid mycelium $(AB) + (ab)$ can diploidise the haploid mycelia (AB) and (ab), and that the diploid mycelium $(Ab) + (aB)$ can diploidise the haploid mycelia (Ab) and (aB). The combinations $(AB) + (ab) \times (AB)$, $(AB) + (ab) \times (ab)$, $(Ab) + (aB) \times (Ab)$, and $(Ab) + (aB) \times (aB)$ were called *legitimate combinations,* for the diploid mycelium in such combinations contains haploid nuclei of the kind required by the haploid mycelium for forming pairs of conjugate nuclei. It was also discovered that diploidisation can be effected in *illegitimate combinations:* $(AB) + (ab) \times (Ab)$, $(AB) + (ab) \times (aB)$, $(Ab) + (aB) \times (AB)$, and $(Ab) + (aB) \times (ab)$.

I found (1931) that, in the illegitimate combination $(AB) + (ab) \times (Ab)$, after diploidisation, the diploidised mycelium (Ab) produced fruit-bodies that gave rise to basidiospores not all exclusively of the (Ab) type, as would have been the case if the mycelium (Ab) had developed by itself and had produced a haploid fruit-body, but of the four types (AB), (ab), (Ab), and (aB), as was found by mating in all possible combinations the mycelia derived from the spores. Thus it was proved that the mycelium $(AB) + (ab)$ had actually diploidised the mycelium (Ab). This first case of the diploidisation of a haploid mycelium by a diploid mycelium in an illegitimate combination came to me as a great surprise.

The Buller phenomenon has been discussed by Rawitscher (1933) in a review of my work; and it has been investigated experimentally by Quintanilha (1933–1939) in *Coprinus lagopus* (his *C. fimetarius*), Chow (1934), a pupil of Dangeard, in *C. lagopus,* Dickson

(1934–1936) in *C. sphaerosporus* and *C. macrorhizus,* Noble (1937) in *Typhula Trifolii,* and Oikawa (1939) in *Stropharia semiglobata* and *Galera tenera.* The work of all these authors, except Oikawa, has been reviewed by Quintanilha (1939). Quintanilha and Oikawa have devoted themselves more especially to finding out exactly what happens to the nuclei when a diploid mycelium diploidises a haploid mycelium in a so-called illegitimate combination.

The explanation of what takes place during the diploidisation of a haploid mycelium by a diploid mycelium in such a Hymenomycete as *Coprinus lagopus* in (1) legitimate combinations and (2) in illegitimate combinations will now be considered.

(1) *Legitimate combinations, e.g.* $(AB) + (ab) \times (ab)$. It can readily be supposed that, after the two mycelia have formed hyphal fusions with one another, one or more (AB) nuclei make their way from the diploid mycelium into the haploid mycelium, divide there, and so provide a sufficient number of (AB) nuclei to supply conjugate mates for all the (ab) nuclei in the growing hyphae of the (ab) mycelium. This explanation was illustrated with a series of diagrams in my *Researches on Fungi* (1931), and it has been accepted by Quintanilha (1933, 1939) and Oikawa (1939).

Dickson (1934–1936) working with *Coprinus sphaerosporus* (a bipolar species) and *C. macrorhizus* (a tetrapolar species) and using mycelia whose nuclei were marked with certain factors, admitted that some of his results obtained with legitimate combinations could be explained in the manner just set forth; but he also found that other results justified the supposition that *both of the nuclei* of the diploid mycelium had moved into the haploid mycelium. Quintanilha (1939), working with *Coprinus fimetarius* (*C. lagopus*) found, in his legitimate combinations, one case only for which the results could be best explained by the double-migration theory. He supposed that, in the case in question, both nuclei of the diploid mycelium had passed into the haploid mycelium and had there formed conjugate pairs thus diploidising the haploid mycelium without the nuclei of this mycelium taking any part in the process.

(2) *Illegitimate combinations, e.g.* $(AB) + (ab) \times (Ab)$. Here neither the (AB) nucleus nor the (ab) nucleus of the diploid mycelium is able to form a conjugate pair with an (Ab) nucleus of

the haploid mycelium because the nuclei (AB) and (Ab) have the factor (A) in common and the nuclei (ab) and (Ab) have the factor (b) in common, and nuclei with one or two factors in common do not attract one another.

Rawitscher (1933), in his review, suggested that diploidisation in such a combination as $(AB) + (ab) \times (Ab)$ is effected by both the (AB) and (ab) nuclei moving from the diploid mycelium into the haploid mycelium and there taking possession of the cytoplasm; and this relatively simple explanation was favoured by myself (1933, Preface), Chow (1934), Dickson (1934–1936), and Oikawa (1939).

Quintanilha has admitted that the explanation just given for diploidisation in illegitimate matings may be the correct one sometimes, but states that in his experiments he has not found any cases that need it; and he has put forward another explanation which he believes is applicable to his findings. This new explanation or hypothesis is as follows. In such an illegitimate combination as $(aB) + (Ab) \times (AB)$, neither the (aB) nucleus nor the (Ab) nucleus can form conjugate mates with an (AB) nucleus. The required nucleus is (ab), and this is formed by an *exchange of chromosomes* between the (aB) nucleus and the (Ab) nucleus of a conjugate pair at a time when these nuclei are dividing in the presence of an (AB) nucleus of the haploid mycelium. After the conjugate nuclei (aB) and (Ab) have nearly divided and are in the anaphase condition (before the formation of nuclear membranes), in one daughter pair of nuclei $(aB) + (Ab)$, near the (AB) nucleus, the (a) and (A) chromosomes of the (aB) and (Ab) nuclei are *exchanged,* with the result that there are formed two nuclei (AB) and (ab) instead of two nuclei (aB) and (Ab). Thus the required nucleus (ab) comes into existence and, by division, can be employed for diploidising the (AB) mycelium.

Quintanilha's theory, as just set forth, was arrived at to explain his experimental data after he had eliminated other theories as untenable, but without the support of any direct observations on the division of nuclei. It therefore cannot be regarded as having been proved.

The idea that a daughter nucleus on one nuclear spindle may exchange a chromosome with another daughter nucleus on another nuclear spindle just before these daughter nuclei become enclosed in

their nuclear membranes may not be readily accepted by those who have studied nuclear division in plants and animals in general. Moreover, the number of experiments to the results of which Quintanilha has applied his hypothesis of chromosome exchange with apparent success are but few in number. Finally, it must be pointed out that Quintanilha's theory does not account for the occurrence of *patchiness* in the diploidised mycelium. This objection to the theory, already raised by Chow (1934), will now be developed in detail.

When investigating the diploidisation of haploid mycelia of *Coprinus lagopus* I devised the method of mating a large haploid mycelium with a very small diploid mycelium. The haploid mycelium was allowed to grow on a dung-agar plate until it was about 6 cm. in diameter. It was then inoculated at its edge at one point with a diploid mycelium only about 1 mm. in diameter. The small diploid mycelium gradually diploidised all the growing hyphae of the haploid mycelium, as was shown by the hyphae changing their mode of branching from obtuse-angled to acute-angled and developing clamp-connexions.

In a small-diploid × large-haploid *legitimate* combination of mycelia of *Coprinus lagopus, e.g.* $(AB) + (ab) \times (ab)$, after diploidisation had been effected, it was seen that the diploid mycelium newly formed all around the periphery of the large diploidised (ab) mycelium was *even* and *regular* in appearance. On the other hand, in a small-diploid × large-haploid *illegitimate* combination, *e.g.* $(AB) + (ab) \times (Ab)$, after diploidisation had been effected, it was observed that the diploid mycelium newly formed all around the periphery of the large diploidised (Ab) mycelium was *irregular* and *patchy* in appearance.

In such a small-diploid × large-haploid *illegitimate* combination as $(AB) + (ab) \times (Ab)$, according to Rawitscher's theory, both (AB) and (ab) nuclei pass from the diploid mycelium into the haploid mycelium (Ab) and there form pairs of conjugate nuclei. One may suppose that the invasion of the (Ab) mycelium by (AB) and (ab) nuclei that displace and replace (Ab) nuclei in the cytoplasm might well cause the diploidised mycelium (Ab) to have an *irregular* and *patchy* appearance.

On the other hand, according to Quintanilha, in such a small-diploid × large-haploid *illegitimate* combination as $(AB) + (ab)$

× (*Ab*), soon after the two mycelia have united by means of hyphal fusions, an (*AB*) nucleus and an (*ab*) nucleus of a conjugate pair of nuclei exchange chromosomes and so produce an (*aB*) nucleus which by division and movement then proceeds to diploidise the large haploid mycelium (*Ab*). Now, theoretically, the (*aB*) nucleus should behave in the same way as the (*aB*) nucleus in the legitimate combination (*Ab*) + (*aB*) × (*Ab*), *i.e.* it should diploidise the large haploid mycelium (*Ab*) in an even and regular manner. But this is not the case; for, as I have observed, the diploid mycelium formed all around the periphery of the diploidised (*Ab*) mycelium is irregular and patchy in appearance.

It will thus be seen that the important fact that, in a small-diploid × large-haploid illegitimate combination, the diploidised mycelium is patchy in appearance instead of even is sufficiently explained by the double-migration theory of Rawitscher but not by the chromosome-exchange theory of Quintanilha.

Rawitscher's double-migration theory of diploidisation in illegitimate diploid × haploid combinations gains support in the results obtained by Oikawa (1939) and Quintanilha (1939) from small-diploid × large-haploid combinations in which the two mycelia belonged to two different *geographical races*.

XXIII. GEOGRAPHICAL RACES AND THE BULLER PHENOMENON

Geographical races in Hymenomycetes were discovered by Kniep in 1920 when working with *Schizophyllum commune*. Among other Hymenomycetes now known to include geographical races are the following:

Panaeolus campanulatus (Vandendries, 1923,a),
Coprinus radians (Vandendries, 1925,a),
Coprinus comatus (Brunswik, 1924),
Coprinus niveus (Brunswik, 1924),
Coprinus lagopus (Hanna, 1925),
Coprinus Rostrupianus (D. Newton, 1926,b),
Fomes pinicola (Mounce, 1929),
Galera tenera (Oikawa, 1939), and
Stropharia semiglobata (Oikawa, 1939).

The geographical races of any one species of Hymenomycetes inhabiting one large region and even regions a long way apart are, as a rule, completely interfertile with one another; but in *Coprinus*

micaceus (Vandendries, 1927, 1928–1929) and *Fomes pinicola* (Mounce, 1929) certain geographical races behave exceptionally in that they are completely infertile with one another. In *Coprinus lagopus,* so far, all the geographical races, even those obtained from such distant regions as western Canada and England, have proved to be completely interfertile (Hanna, 1925).

As an introduction to an account of one of Oikawa's experiments, the nature of completely interfertile geographical races, with *Coprinus lagopus* as an example, will now be explained.

Hanna (1925), in his investigation of the sexuality of *Coprinus lagopus* obtained a number of geographical races, for three of which the localities and kinds of haploid mycelia (haplonts) developed from the haploid basidiospores may be represented as follows:

Race I. At Winnipeg. (A^1B^1), (a^1b^1), (A^1b^1), (a^1B^1).
Race II. At Saskatoon. (A^2B^2), (a^2b^2), (A^2b^2), (a^2B^2).
Race III. At Edmonton. (A^3B^3), (a^3b^3), (A^3b^3), (a^3B^3).

Successful matings of haplonts in any *one* geographical race are achieved only when the haplonts have no factors in common. Thus the only combinations resulting in mutual diploidisation in our three races are for:

Race I. $(A^1B^1) \times (a^1b^1)$ and $(A^1b^1) \times (a^1B^1)$.
Race II. $(A^2B^2) \times (a^2b^2)$ and $(A^2b^2) \times (a^2B^2)$.
Race III. $(A^3B^3) \times (a^3b^3)$ and $(A^3b^3) \times (a^3B^3)$.

Between all the haplonts of any *two* geographical races there is *complete fertility.* Thus, for example, in Races I and II, any of the four haplonts (A^1B^1); (a^1b^1), (A^1b^1), and (a^1B^1) can diploidise any of the four haplonts (A^2B^2), (a^2b^2), (A^2b^2), and (a^2B^2), and *vice versa.*

In a single geographical race in a *legitimate* diploid × haploid combination such as $(A^1B^1) + (a^1b^1) \times (a^1b^1)$, only *one nucleus* of the diploid mycelium, here the (A^1B^1) nucleus, is compatible with the nucleus of the haploid mycelium; but, in a diploid × haploid combination where two geographical races are concerned, such as $(A^1B^1) + (a^1b^1) \times (a^2b^2)$, both of the nuclei of the diploid mycelium, here both the (A^1B^1) nucleus and the (a^1b^1) nucleus, are compatible with the nucleus of the haploid mycelium. Therefore, if we wish, we can say that a diploid × haploid combination involving any two geographical races is *doubly legitimate.*

Oikawa, working with *Galera tenera* obtained two geographical

races which, with symbols like those used above for *Coprinus lagopus,* may be represented thus:

Race I. Locality I. (A^1B^1), (a^1b^1), (A^1b^1), (a^1B^1).
Race II. Locality II. (A^2B^2), (a^2b^2), (A^2b^2), (a^2B^2).

Oikawa inoculated a large haploid mycelium (a^2B^2) of Race II with a small diploid mycelium $(A^1b^1)+(a^1B^1)$ of Race I; and, after diploidisation of the large (a^2B^2) mycelium had been effected, he removed various portions of the diploidised mycelium and grew them separately on culture media. These sub-cultures produced fruit-bodies. The basidiospores of these fruit-bodies were then germinated and the haplonts thus obtained were tested for their genetic constitution by mating with tester mycelia. In one experiment Oikawa obtained two fruit-bodies, one from one piece of the diploidised mycelium and the other from another piece; and subsequent analysis indicated that one piece of the mycelium and its fruit-body must have had the genetic constitution $(A^1b^1)+(a^2B^2)$, and the other piece of the mycelium and its fruit-body must have had the genetic constitution $(a^1B^1)+(a^2B^2)$. Oikawa explained this result very simply; both (A^1b^1) and (a^1B^1) nuclei of the small diploid mycelium passed into the large haploid mycelium (a^2B^2) and, as both the invading nuclei were compatible with the (a^2B^2) nuclei and could form conjugate nuclei with them, one part of the large mycelium (a^2B^2) had been diploidised by (A^1b^1) nuclei and another part by (a^1B^1) nuclei.

Quintanilha's experiments with diploid × haploid combinations where two geographical races were concerned were made with *Coprinus lagopus* (his *C. fimetarius*) and they yielded results similar to those obtained by Oikawa.

The experimental results obtained by Oikawa and Quintanilha indicate that at least in diploid × haploid combinations of geographical races, both the kinds of nuclei in the diploid mycelium can invade the haploid mycelium and take part in the diploidisation process. Since there is double-invasion in diploid × haploid combinations of geographical races, one may argue that there may well be double-invasion in illegitimate diploid × haploid combinations within a single geographical race.

XXIV. THE BULLER PHENOMENON IN RUST FUNGI AND PYRENOMYCETES

A. M. Brown has shown that the Buller phenomenon can be ob-

served also in the Rust Fungi. He found that, in the autoecious species, *Puccinia helianthi* (1932), *Uromyces trifolii hybridi, U. fabae,* and *Phragmidium speciosum* (1940), a diploid mycelium derived from a uredospore is able to diploidise the proto-aecidia formed by a haploid mycelium derived from a basidiospore, with the result that the proto-aecidia become converted into dikaryotic diploid aecidia. Oliviera (1940), working independently, has verified the fact that the Buller phenomenon can be produced experimentally in *Phragmidium speciosum.*

A phenomenon comparable with the Buller phenomenon in Hymenomycetes and Uredinales has been observed in certain Pyrenomycetes with four-spored asci.

In *Neurospora tetrasperma,* Dodge (1931) succeeded in obtaining fertile hybrid ascocarps when he mated certain bisexual (hermaphroditic, totipotent) races derived from normal bisexual ascospores containing nuclei of both sexes with certain unisexual races derived from dwarf spores containing nuclei of one sex only. The hybrid ascocarps in each combination formed in the plate cultures along a line where the two mycelia met.

In *Gelasinospora tetrasperma,* another Pyrenomycete with normal bisexual spores and occasional unisexual dwarf spores, Dowding and Buller (1940) obtained results similar to those of Dodge.

The bisexual mycelia used by Dodge and by Dowding and Buller in their experiments have cells containing a mixture of nuclei of both sexes not arranged in conjugate pairs; and, therefore, one must describe these mycelia *not as diploid* but as *bisexual* or as *sexually heterokaryotic.*

XXV. THE TERM BULLER PHENOMENON RE-DEFINED

Quintanilha (1937), in defining the term *Buller Phenomenon* said: "Nous appelons *Phénomène de Buller,* en hommage au botaniste canadien qui l'a découvert, *le phénomène de la diploïdisation d'un mycélium primaire d'Hyménomycète par un mycélium secondaire.*" By primary mycelium was meant a haploid mycelium and by secondary mycelium a dikaryotic diploid mycelium.

Now that the Buller phenomenon has been found to occur in the Uredinales as well as in the Hymenomycetes and also, in principle, in certain Ascomycetes, Quintanilha's definition requires revision. The facts that must come within the compass of a more compre-

hensive definition are as follows: in the Hymenomycetes, a diploid mycelium diploidises a haploid mycelium; in the Uredinales, a diploid mycelium diploidises the basal (fertile) cells of a haploid proto-aecidium; and, in the Pyrenomycetes, a bisexual mycelium diploidises a haploid oogonium of a proto-perithecium.

If we take into account that diploidisation of haploids by diploids may occur in groups of Basidiomycetes other than the Hymenomycetes and Uredinales and in groups of Ascomycetes other than the Pyrenomycetes; and if we recognise that a dikaryotic diploid mycelium of a Hymenomycete or a Rust Fungus is just as bisexual as a bisexual mycelium of a Pyrenomycete such as *Neurospora tetrasperma* or *Gelasinospora tetrasperma;* then we can define the *Buller phenomenon* as: *in Basidiomycetes and Ascomycetes, the diploidisation of a unisexual mycelium or the unisexual rudiment of a fructification by a bisexual mycelium.*

XXVI. BIOLOGICAL SIGNIFICANCE OF THE DIPLOIDISATION OF HAPLOID MYCELIA BY DIPLOID MYCELIA

The discovery that, in the Higher Fungi, a dikaryotic diploid mycelium can diploidise a haploid mycelium has revealed that a dikaryotic diploid cell has sexual powers denied to a synkaryotic cell.

Doubtless, in the Hymenomycetes, under natural conditions, the diploidising power of dikaryotic diploid mycelia often comes into play and works to the advantage of reproduction (Buller, 1930, 1931). The case of *Coprinus lagopus* may be cited by way of illustration.

Coprinus lagopus is a coprophilous fungus very frequently found on horse dung in fields. The spores liberated from the pilei of its sporophores are carried off by the wind, settle on herbage, and stick there until the herbage is eaten by herbivorous animals. The spores pass through the alimentary canal of horses, etc., and they germinate in the solid excreta soon after this has fallen to the ground. Often, in a single horse-dung ball, a large number of basidiospores of *C. lagopus* are enclosed.

In a freshly deposited horse-dung ball, the spores of *Coprinus lagopus* germinate, the mycelia meet, and diploidisation sets in. In such a dung-ball it must often happen that diploid mycelia meet with haploid mycelia and diploidise them. Such diploidisation

must greatly increase the chances that the mycelial hyphae on the exterior of the dung-ball will produce vigorous dikaryotic diploid fruit-bodies that will liberate many spores of all the four types, (AB), (ab), (Ab), and (aB), rather than pale and feeble haploid fruit-bodies that, as Hanna found (1925), produce and liberate no spores or relatively very few spores and spores of one of the four types only.

XXVII. BIBLIOGRAPHY

AMES, L. M. 1934. Hermaphroditism involving self-sterility and cross-fertility in the Ascomycete *Pleurage anserina*. Mycologia **26**: 392–414.

ARTHUR, J. C. *et al.* 1929. The Plant Rusts (Uredinales). New York.

ASHWORTH, DOROTHY. 1931. *Puccinia malvacearum* in monosporidial culture. Trans. Brit. Myc. Soc. **16**: 177–202.

BAIL, T. 1856. Sclerotium and Typhula. Hedwigia **1**: 96–98.

BARY, A. DE. 1859. Zur Kenntniss einiger Agaricinen. Bot. Zeit. **17**: 386.

———. 1866. Morphologie und Physiologie der Pilze, etc. Leipzig.

———. 1884. Vergleichende Morphologie und Physiologie der Pilze, etc. Leipzig.

BAUCH, R. 1923. Über *Ustilago longissima* und ihre Varietät *macrospora*. Zeitschr. f. Bot. **15**: 241–279.

———. 1926. Untersuchungen über zweisporige Hymenomyzeten. Haploid Parthenogenesis bei *Camarophyllus virgineus*. Zeitschr. f. Bot. **18**: 337–387.

BENSAUDE, MATHILDE. 1918. Recherches sur le cycle évolutiv et la sexualité chez les Basidiomycètes. Thesis, Nemours. pp. 1–156.

BESSEY, E. A. 1935. A Text-book of Mycology. Philadelphia.

BIGGS, ROSEMARY. 1938. Cultural studies in the Thelephoraceae and related fungi. Mycologia **30**: 64–78.

BLACKMAN, V. H. 1904. On the fertilization, alternation of generations, and general cytology of the Uredineae. Ann. Bot. **18**: 323–373.

BOSS, G. 1927. Beiträge zur Zytologie der Ustilagineen. Planta **3**: 619–622.

BREFELD, O. 1877, 1888, 1889. Untersuchungen über Pilze. Leipzig. Heft III, Heft VII, Heft VIII.

BRODIE, H. J. 1934. The occurrence and function of oidia in the Hymenomycetes. Am. Journ. Bot. **23**: 309–327.

BROWN, A. M. 1932. Diploidization of haploid by diploid mycelium of *Puccinia Helianthi*. Nature **130**: 777.

———. 1940. The sexual behaviour of several plant Rusts. Can. Journ. Res. **18**: 18–25.

———. 1941. Studies on the perennial rust *Puccinia minussensis*. Can. Journ. Res. **19**: 75–79.

BRUNSWIK, H. 1924. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*. Bot. Abhnd. (Goebel). **5**: 1–152.

BUHR, H. 1932. Untersuchungen über zweisporige Hymenomyceten. Archiv f. Protistenkunde **77**: 125–151.

BULLER, A. H. R. 1909. Researches on Fungi. I. London.

———. 1922. Researches on Fungi. II. London.

———. 1924. Researches on Fungi. III. London.

———. 1930. The biological significance of conjugate nuclei in *Coprinus lagopus* and other Hymenomycetes. Nature **126**: 686–689.

———. 1931. Researches on Fungi. IV. London.

———. 1933. Researches on Fungi. V. London.

————. 1934. Researches on Fungi. VI. London.
————. 1938. Fusions between flexuous hyphae and pycnidiospores in *Puccinia graminis*. Nature **141**: 33.
————, AND A. M. BROWN. 1941. Urediospores as the origin of systemic mycelia in *Puccinia suaveolens*. (Abstr.) Phytopathology **31**: 4.
CHILTON, ST. J. P. 1938. The occurrence of lysis in certain crosses of *Ustilago zeae*. (Abstr.) Phytopathology **28**: 5.
CHOW, C. H. 1934. Contribution à l'étude du développement des Coprins. Le Botaniste **26**: 89–232.
CHRISTENSEN, J. J. 1931. Studies on the genetics of *Ustilago zeae*. Phytopath. Zeitschr. **4**: 129–188.
————, AND H. A. RODENHISER. 1940. Physiologic specialisation and genetics of the Smut Fungi. Bot. Rev. **6**: 389–425.
CLAUSSEN, P. 1912. Zur Entwicklungsgeschichte der Ascomyceten. *Pyronema confluens*. Zeitschr. f. Bot. **4**: 1–64.
COLSON, BARBARA. 1935. The cytology of the mushroom *Psalliota campestris* Quel. Ann. Bot. **49**: 1–18.
COUCH, J. N. 1938. The genus Septobasidium. Univers. North Carolina Press.
CRAIGIE, J. H. 1927, a. Experiments on sex in the Rust Fungi. Nature **120**: 116–117.
————. 1927, b. Discovery of the function of the pycnia of the Rust Fungi. Nature **120**: 765–767.
————. 1940. The origin of physiologic races of Rust Fungi through hybridization. In: The Genetics of Pathogenic Organisms. The Science Press. Publ. no. 12. Am. Ass. Adv. Sci. pp. 66–72.
DERX, H. G. 1925. L'hétérothallie dans le genre *Penicillium* (Note préliminaire). Bull. Soc. Myc. France **41**: 375–381.
————. 1930. Étude sur les Sporobolomycètes. Ann. Mycologici **27**: 1–23.
DICKINSON, S. 1927. Experiments on the physiology and genetics of the Smut Fungi—Hyphal Fusion. Proc. Roy. Soc. London. B, **101**: 126–136.
DICKSON, H. 1934. Studies in *Coprinus sphaerosporus*. I. The pairing behaviour and the characteristics of various haploid and diploid strains. Ann. Bot. **48**: 527–547.
————. 1935. Studies in *Coprinus sphaerosporus*. II. The inheritance of various morphological and physiological characters. Ann. Bot. **49**: 181–204.
————. 1936, a. Studies in *Coprinus sphaerosporus*. III. The inheritance of factors affecting the growth-rates at different temperatures of certain strains. Ann. Bot. **50**: 219–246.
————. 1936, b. Observations on inheritance in *Coprinus macrorhizus* (Pers.) Rea. Ann. Bot. **50**: 719–734.
DODGE, B. O. 1920. The life-history of *Ascobolus magnificus*. Mycologia **12**: 115–134.
————. 1927. Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. Journ. Agr. Res. **35**: 289–305.
————. 1932. Crossing hermaphroditic races of Neurospora. Mycologia **24**: 7–13.
————. 1934. A lethal for ascus abortion in Neurospora. Mycologia **26**: 360–376.
————. 1935. A recessive lethal factor for ascospore formation in Neurospora. Bull. Torrey Bot. Club **62**: 117–128.
————. 1936, a. Interspecific hybrids involving factors for ascus abortion in Neurospora. Am. Journ. Bot. **23**: 555–561.
————. 1936, b. Spermatia and nuclear migration in *Pleurage anserina*. Mycologia **28**: 284–291.

———. 1939, a. Some problems in the genetics of the Fungi. Science **90**: 379–385.

———. 1939, b. A new dominant lethal in Neurospora. Journ. Hered. **30**: 467–470.

———. 1940. Second-division segregation and crossing-over in the Fungi. Bull. Torrey Bot. Club **67**: 467–476.

———, AND LULU O. GAISER. 1926. The question of nuclear fusions in the Blackberry Rust. Journ. Agric. Res. **32**: 1008–1012, 1022–1023.

———, AND BERNICE SEAVER. 1938. The combined effects of the dominant and recessive lethals for ascus abortion in Neurospora. Am. Journ. Bot. **25**: 156–166.

DOWDING, E. S. 1931, a. The sexuality of the normal, giant, and dwarf spores of *Pleurage anserina* (Ces.) Kunze. Ann. Bot. **45**: 1–15.

———. 1931, b. The sexuality of *Ascobolus stercorarius* and the transportation of the oidia by mites and flies. Ann. Bot. **45**: 621–637.

———. 1933. Gelasinospora, a new genus of Pyrenomycetes with pitted spores. Can. Journ. Res. **9**: 294–305.

———, AND A. H. R. BULLER. 1940. Nuclear migration in Galasinospora. Mycologia **32**: 471–488.

DRAYTON, F. L. 1932. The sexual function of the microconidia in certain Discomycetes. Mycologia **24**: 345–348.

———. 1934. The sexual mechanism of *Sclerotinia Gladioli.* Mycologia **26**: 46–72.

———. 1937. The perfect stage of *Botrytis convoluta.* Mycologia **29**: 305–318.

EMMONS, C. W. 1935. The ascocarps in species of Penicillium. Mycologia **27**: 128–150.

FALCK, R. 1909. Hausschwammforschungen. Heft 3. Die Lenzites-Fäule des Coniferenholzes. Jena.

———. 1912. *Ibid.* Heft 6. Die Merulius-Fäule des Bauholzes. Jena.

FLOR, H. H. 1932. Heterothallism and hybridization in *Tilletia tritici* and *T. levis.* Journ. Agr. Res. **44**: 49–58.

GÄUMANN, E. A., AND C. W. DODGE. 1928. Comparative Morphology of Fungi. New York.

GOLDSCHMIDT, V. 1928. Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* (Pers.)). Ein Beitrag zur Frage der parasitären Spezialisierung. Zeitschr. f. Bot. **21**: 1–90.

GREGORY, P. H. 1938. *Sclerotinia polyblastis* n. sp. on Narcissus, the perfect stage of *Botrytis polyblastis* Dowson. Trans. Brit. Myc. Soc. **22**: 201–203.

GREIS, H. 1937. Entwicklungsgeschichtliche Untersuchungen an Basidiomyceten. III. Entwicklung der Wirbelschnallen bei *Coniophora cerebella.* Jahrb. f. wiss. Bot. **84**: 740–763.

GROVE, W. B. 1913. The British Rust Fungi. Cambridge Univ. Press.

GROVES, J. W., AND F. L. DRAYTON. 1939. The perfect stage of *Botrytis cinerea.* Mycologia **31**: 485–489.

GUILLIERMOND, A. 1927. Étude cytologique et taxonomique sur les levures du genre Sporobolomyces. Bull. Soc. Myc. France **43**: 245–258.

———. 1931. Recherches sur l'homothallisme chez les Levures. Rev. Gen. Bot. **43**: 49–93.

———. 1937. La sexualité, le cycle de développement, la phylogénie, et la classification des Levures d'après les travaux récents. pp. 1–72. Paris.

———. 1940. Sexuality, developmental cycle, and phylogeny of Yeasts. Bot. Rev. **6**: 1–24.

HANNA, W. F. 1925. The problem of sex in *Coprinus lagopus.* Ann. Bot. **39**: 431–457.

———. 1928. Sexual stability in monosporous mycelia of *Coprinus lagopus*. Ann. Bot. **42**: 379–388.

———. 1929. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopathology **19**: 415–442.

———. 1932. The odor of Bunt spores. Phytopathology **22**: 978–979.

HANSEN, E. C. 1891. Undersøgelser over alcoholgjaersvampenes fysiologi og morfologi. Medd. fra Carlsberg Lab. **3**: 53. Cited from Winge. 1935.

HARDER, R. 1926. Microchirurgische Untersuchungen über die geschlechtliche Tendenz der Paarkerne des homothallischen *Coprinus sterquilinus*. Planta **2**: 446–453.

HARTIG, R. 1874. Wichtige Krankheiten der Waldbäume. Berlin.

———. 1878. Die Zersetzungserscheinungen des Holzes. Berlin.

———. 1885. Die Zerstörung des Bauholzes durch Pilze. I. Der ächte Hausschwamm (*Merulius lacrymans*). Berlin.

HARTMANN, M. 1918. Theoretische Bedeutung und Terminologie der Vererbungserscheinungen bei haploiden Organismen. Zeitschr. f. Ind. Abst. u. Vererb. **20**: 1–26.

———. 1929. Verteilung, Bestimmung, und Vererbung des Geschlechts bei den Protisten und Thallophyten. Handbuch der Vererbungswissenschaft. Lief. 9 (II,E): 1–115.

———. 1939. Geschlecht und Geschlechtsbestimmung im Tier und Pflanzenreich. Berlin.

HELDMAIER, CLARA. 1929. Über die Beeinflussbarkeit der Sexualität von *Schizophyllum commune* (Fr.) und *Collybia velutipes* (Curt.). Zeitschr. f. Bot. **22**: 161–220.

HIRMER, MAX. 1920. Zur Kenntnis der Vielkernigkeit der Autobasidiomyzeten, I. Zeitschr. f. Bot. **12**: 668–669.

HOFFMANN, H. 1856. Die Pollinarien und Spermatien von Agaricus. Bot. Zeit. **14**: 156.

HOLTON, C. S. 1931. Hybridization and segregation in the Oat Smuts. Phytopathology **21**: 835–842.

JACKSON, H. S. 1931. Present evolutionary tendencies and the origin of life cycles in the Uredinales. Mem. Torrey Bot. Club **18**: 1–108.

———. 1935. The nuclear cycle in *Herpobasidium filicinum* with a discussion of the significance of homothallism in Basidiomycetes. Mycologia **27**: 553–572.

JOHNSON, T., AND MARGARET NEWTON. 1933. Hybridization between *Puccinia graminis tritici* and *Puccinia graminis avenae*. Proc. World's Grain Exhib. and Conference, Canada **2**: 219–223.

———, AND ———. 1938. The origin of abnormal rust characteristics through the inbreeding of physiological races of *Puccinia graminis tritici*. Can. Journ. Res. C, **16**: 38–52.

———, AND ———. 1940, a. Crossing and selfing studies with physiological races of Oat Stem Rust. Can. Journ. Res. C, **18**: 54–67.

———, AND ———. 1940, b. Mendelian inheritance of certain pathogenic characters of *Puccinia graminis tritici*. Can. Journ. Res. C, **18**: 599–611.

———, AND ——— AND A. M. BROWN. 1934. Further studies of inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. Sci. Agr. **14**: 360–373.

KERL, I. 1937. Über Regenerationsversuche an Fruchtkörpern und andere entwicklungsphysiologische Untersuchungen bei *Pyronema confluens*. Zeitschr. f. Bot. **31**: 129–174.

KLIOUSHNIKOVA, E. S. 1938. Sexual character in cultivated mushroom (*Psalliota campestris* Fr.). In Russian with English summary. Bull. Soc. Nat. Moscou n.s. **47**: 30–38.

———. 1939. The wild *Psalliota campestris*, its sexual character and its

relation to the cultivated mushroom. In Russian with English summary. Bull. Soc. Nat. Moscou n.s. **48**: 53–58.

KLUYVER, A. J., AND C. B. VAN NIEL. 1924. Über Spiegelbilder erzeugender Hefearten und die neue Hefengattung Sporobolomyces. Centralbl. f. Bakt. etc. Abt. 2. **63**: 1–20.

KNIEP, H. 1915. Beiträge zur Kenntnis der Hymenomyzeten. III. Zeitschr. f. Bot. **7**: 369–398.

————. 1920. Über morphologische und physiologische Geschlechtsdifferenzierung (Untersuchungen an Basidiomyzeten). Verhandl. Phys.-Med. Ges. Würzburg. **46**: 1–18. (Report of lecture given Nov. 27, 1919).

————. 1922. Über Geschlechtsbestimmung und Reduktionsteilung (Untersuchungen an Basidiomyzeten). *Ibid.* **47**: 1–29.

————. 1923. Über erbliche Änderungen von Geschlechtsfactoren bei Pilzen. Zeitschr. f. Indukt. Abstammungs- und Vererbungslehre **31**: 170–183.

————. 1928. Die Sexualität der niederen Pflanzen. Jena.

————. 1929, a. Vererbungserscheinungen bei Pilzen. Bibliogr. Genet. **5**: 371–478.

————. 1929, b. Die "multipolare" Sexualität der Hymenomyzeten und deren Deutung durch Hartmann. Zeitschr. f. Bot. **22**: 266–273.

KRUIS, K., AND J. ŠATAVA. 1918. O vývoji a klíčeni spór jakož i sexualitě kvasinek. V Praze. (67 pp. with 24 plates). Cited from Winge. 1935.

KÜHNER, R. 1926. Contribution à l'étude des Hymenomycètes et spécialement des Agaricacés. Le Botaniste **17**: 1–224.

————. 1927. Étude cytologique de l'hymenium de *Mycena galericulata* Scop. Le Botaniste **18**: 168–176.

KURSANOV, L. 1922. Recherches morphologiques et cytologiques sur les Urédinées. Bull. Soc. Nat. Moscou. n.s. (1917). **31**: 1–129.

LAMB, I. M. 1935. The initiation of the dikaryophase in *Puccinia Phragmitis* (Schum.) Körn. Ann. Bot. **49**: 403–438.

LAMBERT, E. B. 1929. The production of normal sporophores in monosporous cultures of *Agaricus campestris*. Mycologia **21**: 333–335.

————. 1938. Principles and problems of mushroom culture. Bot. Rev. **4**: 397–426.

LASKARIS, T. 1939. The occurrence of lysis in certain crosses of *Sphacelotheca sorghi*. (Abst.) Phytopathology **29**: 14.

LEHFELDT, W. 1923. Über die Entstehung des Paarkernmyzels bei heterothallischen Basidiomyzeten. Hedwigia **64**: 13–51.

LEVINE, M. N., AND R. U. COTTER. 1931. A synthetic production of *Puccinia graminis hordei* F. and J. Phytopathology **21**: 107.

LINDEGREN, C. C. 1933. The genetics of Neurospora—III. Pure bred stocks and crossing-over in *N. crassa*. Bull. Torrey Bot. Club **60**: 133–154.

————. 1936, a. The structure of the sex-chromosomes of *Neurospora crassa*. Journ. Hered. **28**: 251–259.

————. 1936, b. A six-point map of the sex-chromosome of *Neurospora crassa*. Jour. Genet. **32**: 243–256.

————, AND G. LINDEGREN. 1939. Non-random crossing-over in the second chromosome of *Neurospora crassa*. Genetics **24**: 1–7.

LINDER, D. H. 1940. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia **32**: 419–447.

LINDFORS, T. 1924. Studien über den Entwicklungsverlauf bei einigen Rostpilzen aus zytologischen und anatomischen Gesichtspunkten. Svensk. Bot. Tidskr. **18**: 34–37.

MACRAE, RUTH. 1937. Interfertility phenomena of the American and European forms of *Panus stypticus* (Bull.) Fries. Nature **139**: 674.

————. 1941. Personal communication.

MAIRE, R. 1902. Recherches cytologiques et taxonomiques sur les Basidiomycètes. Bull. Soc. Myc. France **18**(4): 1–209 (sep. paged).

————. 1912. Myc. Centralbl. **1**: 214.

MANUEL, MLLE. J. 1939. Sur l'hétérothallisme du *Saccharomycodes Ludwigii.* Compt. Rend. Acad. Sci. Paris **209**: 119–221.

MOREAU, MME. F. 1911. Sur l'existence d'une forme écidienne uninucléée. Bull. Soc. Myc. France **27**: 489–493.

————. 1914. Les phénomènes de la sexualité chez les Urédinées. Le Botaniste **13**: 177–188.

————. 1915. Note sur la variété uninucléée de l' *Endophyllum Euphorbiae* (DC.) Winter. Bull. Soc. Myc. France **31**: 68–70.

MOREAU, F., AND MME. F. MOREAU. 1918. L'écidiospore de l' *Endophyllum Euphorbiae-sylvaticae* (DC.) Winter est-elle le siège d'une karyogamie? Bull. Soc. Myc. France **33**: 97–99.

————, AND ————. 1919. Les Urédinées du groupe Endophyllum. Bull. Soc. Bot. France **66**: 27–33.

MOUNCE, IRENE. 1921. Homothallism and the production of fruit-bodies by monosporous mycelia in the genus *Coprinus.* Trans. Brit. Myc. Soc. **7**: 198–217.

————. 1922. Homothallism and heterothallism in the genus *Coprinus.* Trans. Brit. Myc. Soc. **7**: 256–269.

————. 1929. Studies in forest pathology. II. The biology of *Fomes pinicola* (Sw.) Cooke. Dom. Canada Dept. Agr. Bull. III.

NEWTON, DOROTHY E. 1926, a. The distribution of spores of diverse sex on the hymenium of *Coprinus lagopus.* Ann. Bot. **40**: 891–917.

————. 1926, b. The bisexuality of individual strains of *Coprinus Rostrupianus.* Ann. Bot. **40**: 105–128.

NEWTON, MARGARET, AND T. JOHNSON. 1927. Color mutations in *Puccinia graminis tritici* (Pers.) Erikss. and Henn. Phytopathology **17**: 711–725.

————, AND ————. 1940. Variation and hybridization in *Puccinia graminis.* Proc. Third Internat. Congr. Microbiology. New York. p. 544.

————, AND ————, AND A. M. BROWN. 1930. A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici.* Sci. Agr. **10**: 721–731.

————, AND ————, AND ————. 1930. A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici.* Sci. Agr. **10**: 775–798.

NOBLE, MARY. 1937. The morphology and cytology of *Typhula Trifolii.* Ann. Bot. Ser. 2. **1**: 67–98.

NOBLES, MILDRED K. 1935. Conidial formation, mutation, and hybridization in *Peniophora Allescheri.* Mycologia **27**: 286–301.

OIKAWA, K. 1939. Diploidisation and fruit-body formation in the Hymenomycetes. Sci. Reports Tôhoku Imp. Univers. **14**: 245–260.

D'OLIVIERA, B. 1939. Aspectos actuais do problema das Ferrugens. Palestras Agronómicas **2**: 1–77.

OORT, A. J. P. 1930. Die Sexualität von *Coprinus fimetarius.* Rec. Trav. Bot. Neerlandais **27**: 85–148.

POIRAULT, G., AND M. RACIBORSKI. 1895. Sur les noyaux des Urédinées. Journ. de Bot. **9**: 382.

POPP, W., AND W. F. HANNA. 1935. Studies on the physiology of the Oat Smuts. Sci. Agr. **15**: 424–434.

QUINTANILHA, A. 1933. Le problème de la sexualité chez les Champignons. Bol. Soc. Broter. Sér. 2. **8**: 1–100.

————. 1935. Cytologie et génétique de la sexualité chez les Hyménomycètes. *Ibid.* **10**: 289–386.

————. 1937. Contribution à l'étude génétique du phénomène de Buller. Compt. Rend. Acad. Sci. Paris **205**: 745.

———. 1938. Deuxième contribution à l'étude génétique du phénomène de Buller. Compt. Rend. Soc. Biol. **127**: 1245.
———. 1938. Troisième contribution à l'étude génétique du phénomène de Buller. *Ibid.* **129**: 730.
———. 1939. Étude génétique du phénomène de Buller. Bol. Soc. Broter. Sér. 2. **13**: 425–486.
———, AND S. BALLE. 1938. Étude génétique des phénomènes de nanisme chez les Hyménomycètes. Compt. Rend. Soc. Biol. **129**: 191.
———, AND ———. 1940. Étude génétique des phénomènes de nanisme chez les Hyménomycètes. Bol. Soc. Broter. Sér. 2. **14**: 17–46.
RAWITSCHER, F. 1922. Beiträge zur Kenntnis der Ustilagineen. II. Zeitschr. f. Bot. **14**: 274–295.
———. 1933. Besprechungen. Zeitschr. f. Bot. **26**: 134–136.
RENAUD, J. 1937. Sur un *Saccharomyces* présentant dans sa sexualité de curieuses anomalies dûes à un état dicaryotique de ses zygospores. Compt. Rend. Acad. Sci. Paris **204**: 1274–1276.
———. 1938. Sur l'existence du dicaryon chez un *Saccharomyces* isolé du vin. Compt. Rend. Acad. Sci. Paris **206**: 1397–1399.
RENNER, O. 1916. Zur Terminologie des Generationswechsels. Biol. Zentralbl. **36**: 337–374.
RUTTLE, MABLE L. (Mrs. Nebel). 1934. Studies on Barley smuts and on loose smut of Wheat. N.Y. (Geneva) Agric. Exp. Station Tech. Bull. **221**: 1–39.
SAPPIN-TROUFFY, P. 1896. Recherches histologiques sur la famille des Urédinées. Le Botaniste **5**: 184–187.
SARAZIN, A. 1938. Évolution nucléaire de la baside et des basidiospores dans *Agaricus campestris* (var. cultivée). Compt. Rend. Acad. Sci. Paris **206**: 275–278.
———. 1939. Cultures monospermes d'*Agaricus campestris* var. cultivée. Compt. Rend. Acad. Sci. Paris **208**: 2015–2017.
SASS, J. E. 1929, a. A cytological basis for homothallism and heterothallism in the Agaricaceae. Am. Journ. Bot. **16**: 663–701.
———. 1929, b. A cytological study of a bispored form of *Psalliota campestris*. Papers Mich. Acad. Sci. **9**: 287–298.
———. 1936. Cytology of spore germination in the bispored form of *Psalliota campestris*. Mycologia **28**: 431–432.
ŠATAVA, J. 1918. O redukovaných formách kvasinek. V Praze. (48 pp. with 3 plates). Cited from Winge, 1935.
———. 1934. Les formes sexuelles et asexuelles des Levures et leur pouvoir fermentatif. III Congrès internat. technique et chimique des industries agricoles. Paris. (8 pp. with 2 plates). Cited from Winge, 1935.
SEYFERT, R. 1927. Über Schnallenbildung im Paarkernmyzel der Brandpilze. Zeitschr. f. Bot. **19**: 577–601.
SHEAR, C. L., AND B. O. DODGE. 1927. Life histories and heterothallism of the Red Bread-Mold Fungi of the *Monilia sitophila* group. Jour. Agr. Res. **34**: 1019–1042.
SLEUMER, H. O. 1932. Über Sexualität und Zytologie von *Ustilago zeae* (Beckm.) Unger. Zeitsch. f. Bot. **25**: 209–263.
SMITH, A. H. 1934. Investigations of two-spored forms in the genus Mycena. Mycologia **26**: 305–331.
STAKMAN, E. C., AND J. J. CHRISTENSEN. 1927. Heterothallism in *Ustilago zeae*. Phytopathology **17**: 827–834.
STRASBURGER, E. 1905. Typische und allotypische Kernteilung. Ergebnisse und Eröterungen. Jahrb. f. wiss. Bot. **42**: 1–71.
———. 1907. Die Ontogenie der Zelle seit 1875. Progressus Rei Botanicae **1**: 1–138.

TAPKE, V. H. 1932. An undescribed loose smut of Barley. (Abstr.) Phytopathology **22**: 869–870.

————. 1935. A study of the cause of variability in response of Barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Res. **51**: 491–508.

THAXTER, R. 1896–1931. Contribution toward a monograph of the Laboulbeniaceae. Mem. Amer. Acad. Arts and Sci. 1896, **12**: 187–429; 1908, **13**: 217–469; 1924, **14**: 309–426; 1926, **15**: 431–580; 1931, **16**: 1–435.

TULASNE, L.-R., AND C. 1861. Selecta Fungorum Carpologia. I: 115 (W. B. Grove's translation I: 117). Paris.

VANDENDRIES, R. 1923, a. Nouvelles recherches sur la sexualité des Basidiomycètes. Bull. Soc. Roy. Bot. Belg. **56**: 73–97.

————. 1923, b. Recherches sur le déterminisme sexuel des Basidiomycètes. Mém. Acad. Belg. Cl. des Sci. Sér. 2. **5**: 1–98.

————. 1924. Recherches expérimentales sur la bipolarité sexuelle des Basidiomycètes. Bull. Soc. Bot. Belg. **57**: 75–78.

————. 1925, a. Contribution nouvelle à l'étude de la sexualité des Basidiomycètes. La Cellule **35**: 129–155.

————. 1925, b. L'hétéro-homothallisme dans le genre Coprinus. Bull. Soc. Roy. Bot. Belg. **57**: 139–146.

————. 1927. Les mutations sexuelles, l'hétéro-homothallisme et la stérilité entre races géographiques de *Coprinus micaceus*. Mém. Acad. Roy. Belg. (Octavo) **9**: (fasc. 3) 1–50.

————. 1928–1929. Nouvelles recherches expérimentales sur le comportement sexuel de *Coprinus micaceus*. Mém. Acad. Roy. Belg. (Quarto) **9**: (fasc. 1) 1–128; (fasc. 7) 1–117.

————. 1930, a. La conduite sexuelle des Hymenomycètes interpretée par les theories de Hartmann concernant la bisexualité et la relativité sexualle. Bull. Acad. Roy. Belg. **16**: 1213–1234.

————. 1930, b. Conduite sexuelle de *Psathyrella disseminata* et essais de détermination des valeurs relatives des réalisateurs sexuels selon Hartmann. Bull. Acad. Roy. Belg. **16**: 1235–1249.

————, AND P. MARTENS. 1932. Oidies haploïdes et diploïdes sur mycélium diploïde chez *Pholiota aurivella* Batsch. Bull. Acad. Roy. Belg. **18**: 468–472.

WAHRLICH, W. 1893. Zur Anatomie der Zelle bei Pilzen und Fadenalgen. Scripta Bot. Horti Univers. Imp. Petropolitanae **4**: 101–155.

WAKAYAMA, K. 1930. Contributions to the cytology of Fungi. I. Chromosome number in Agaricaceae. Cytologia **1**: 369–388.

WAKEFIELD, E. M. 1909. Über die Bedingungen der Fruchtkörperbildung, sowie das Auftreten fertiler und steriler Stämme bei Hymenomyceten, Naturw. Zeitschr. f. Forst- und Landwirtschaft **7**: 521–551.

WINGE, Ö. 1935. On haplophase and diplophase in some Saccharomycetes. Compt. Rend. Lab. Carlsberg. Sér. Phys. **21**: 77–112.

————, AND O. LAUSTSEN. 1937. On two types of spore germination, and on genetic segregations in *Saccharomyces* demonstrated through single-spore cultures. Compt. Rend. Lab. Carlsberg. Sér. Phys. **22**: 99–119.

————, AND ————. 1938. Artificial species hybridization in Yeast. *Ibid.* **22**: 235–247.

————, AND ————. 1939, a. *Saccharomycodes Ludwigii* Hansen, a balanced heterozygote. *Ibid.* **22**: 257–373.

————, AND ————. 1939, b. On 14 new yeast types produced by hybridization. *Ibid.* **22**: 337–355.

ZATTLER, F. 1924. Vererbungsstudien an Hutpilzen (Basidiomyceten). Zeitschr. f. Bot. **16**: 433–499.

THE BOTANICAL REVIEW

VOL. VII SEPTEMBER, 1941 No. 9

THE CHEMISTRY OF THE CHROMOSOMES

ADDISON GULICK
University of Missouri

To a greater extent than any other part of the living cell, the chromosomes challenge the curiosity of every person who seeks to know the fundamental chemistry of the processes of life. The chemical nature of the genes, unavailable for direct chemical determination, is best hinted at by the constitution of the chromosomes of which they are part, and from which they draw their substance. The possibility of gene reduplication doubtless depends upon the existence of these microscopic reservoirs of material which we call chromosomes. And very possibly it may be through them that the genes, individually too small to produce their effects directly, obtain the chemical amplification of their action that makes them adequate to their tasks.

Our knowledge of chromosome chemistry comes partly from the macrochemical analysis of preparations of nuclei, partly from staining reactions, optical observations and physical tests that, taken by themselves, give far from satisfactory insight, and only in small part from actual microchemical determinations. Arrival at sound conclusions has been through the tedious process of correlating information from the most diverse kinds of sources and experiments performed in the most diverse types of laboratories. On account of the greater ease of manipulation of soft tissues, a high proportion of the investigations has been upon zoological materials. But our knowledge of materials from plant sources is also increasing, and bringing to light the fact that the parallelism between plant and animal nuclei is definitely closer than had earlier been supposed.

ORGANIC ANALYSIS OF NUCLEI

The first chemical analyses of nuclei were carried out by Friedrich Miescher (81) in 1869, published 1871. He obtained his material from pus cells by digesting away the cytoplasm with pepsin-

HCl, or by disintegrating it with weakly acidulated ether-water. In this way he came to the discovery of the substance which he called nuclein, known to us now as nucleoproteins.

The second step was also due to Miescher, 1874, in his article on the spermatozoa of various vertebrates (82). This presented analyses of salmon sperm, bull sperm, and cursory examinations of material from several other vertebrates. The discovery of the salmon protamine was here first announced (82, 83), and the thesis that the salmon sperm nuclei consisted of a salt-like combination of protamine and nuclein (*viz.*, nucleic acid, in this case). A sulphur-rich component, reported in Miescher's first paper, was encountered again in the analysis of bull sperm nuclei. Thus, Miescher's early discoveries comprise:

(*a*) Nucleic acid, present in all the samples of nuclei.
(*b*) Protamine, in salmon sperm, supposed by Miescher to be non-protein.
(*c*) A sulphur compound in mammalian nuclei, judged by Miescher to be non-protein.
(*d*) Protein, less aberrant than (*b*) and (*c*), doubtless to be interpreted as histone, present chiefly where protamine is absent.

The next important event in this succession was Kossel's study of histone (50) and his characterization of it as a basic protein found in nuclei in a salt-like combination with nucleic acid. The source of his material was separated nuclei of avian red blood corpuscles. The name "histone" comes from him (1884).

After these early fundamental studies there came a period in which progress was made chiefly in our understanding of the structural chemistry and colloid physics of the three substances, nucleic acid, protamine, histone.

Studies of nucleic acids by Schmiedeberg (104), Kossel (48, 49, 51, 52, 55), Walter Jones (42) and many others culminated in Levene's elucidation of their structural chemistry, and especially, so far as we are concerned, in his clarification of the structure of thymonucleic acid. Our present information designates this acid or a close isomere as the nucleic acid component in the chromosomes of all animals and of a great many plants. The evidence is definite in fish sperm (62), calf thymus (60, 62), the nuclear component of pea embryo (45) and rye germ (27), and it is inferen-

tially strong, on the basis of the Feulgen stain, among numerous animals and various plants (9, 24, 28, 90, 123). Levene and collaborators (61–64) discovered the previously unknown desoxyribose, a peculiar 5-carbon sugar radical which occupies the central position in each of the four mononucleotides that go to make the

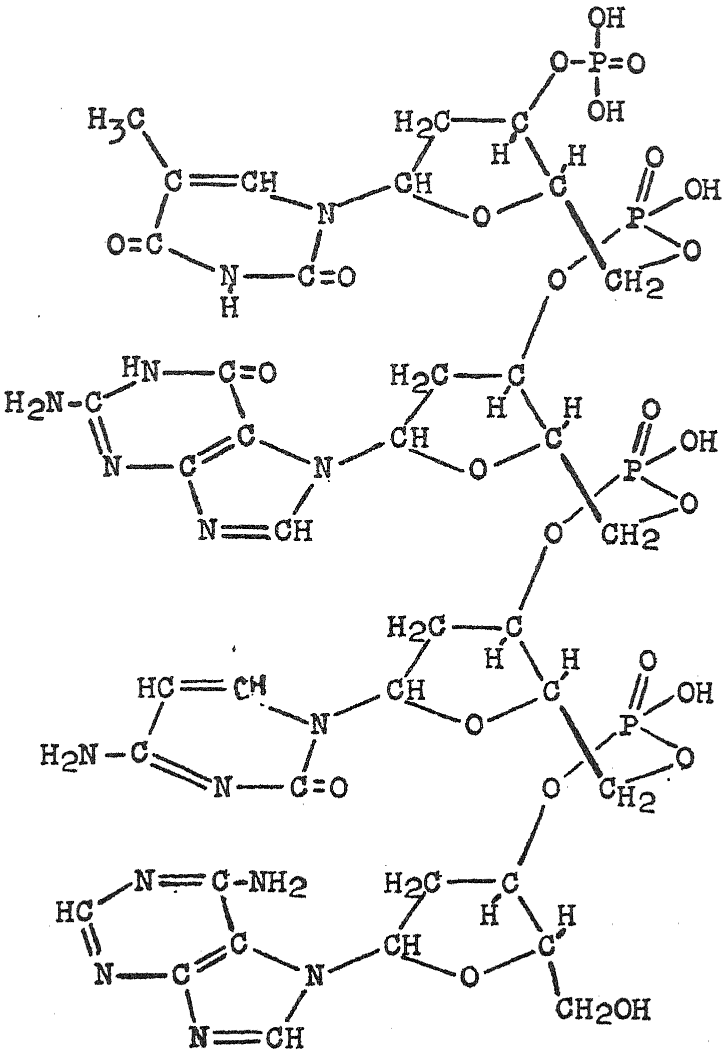


FIG. 1. Thymonucleic acid.

completed tetranucleotides of thymonucleic acid. To the sugar is attached by a sort of glucoside bond the characteristic purine or pyrimidine base, and also by an ester bond the orthophosphoric acid. Thus, each mononucleotide is the threefold compound:

$$\begin{array}{c} \qquad\qquad\qquad\qquad\qquad\quad OH \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \text{Base—Sugar—O—P=O} \\ \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\quad OH \end{array}$$

and the complete minimum or tetranucleotide molecule of thymonucleic acid must have the structural plan that we indicate in Fig. 1. According to Levene (62), this molecule has four strongly ionized acidic hydrogens and one that is very weak. But most investigators conclude that in its natural condition it is a huge super-molecule having one replaceable hydrogen for each mononucleotide. The four aromatic bases comprise two purines, adenine and guanine, and two pyrimidines, thymine and cytosine. Purines and pyrimidines are believed to alternate with each other, but beyond that it is not known in what order.

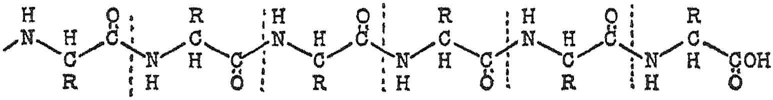


FIG. 2. Polypeptide chain.

The protamines (85, 11, 12, 50, 53, 56, 119, 120) are now recognized to be small polypeptides, which is to say, a sort of miniature protein molecule, there being but 15 to 30 amino-acid residues to the molecule. A high proportion of the amino-acids, frequently two out of every three, are either arginine or (less frequently) some other basic amino-acid, with a result that the total molecule is basic and as such is readily capable of forming a salt with nucleic acid. The occurrence of protamines seems to be limited to the ripe male cells of certain bony fishes (56). The same cells before ripening contain histone but no protamine.

Histones (39, 11, 12, 23, 53, 58, 59), first observed in the red blood corpuscle nuclei of birds, are believed to be the usual type of protein component in the nucleoproteins of animal chromosomes. They have much greater resemblance to the ordinary proteins, possessing a minimal molecular weight of 14,000 (23) or more and

containing a goodly assortment of the different amino-acid varieties. The basic residues are much in evidence, giving to the histones a somewhat basic iso-electric point which fits them to enter into salt-like combinations with nucleic acid. Many investigators (11, 55) declare that their combination with nucleic acid is of two sorts: (*a*) histone nucleate, quickly and easily broken apart by either acid or base; and (*b*) histone-nucleic acid, a somewhat firmly bound compound with acidic characters less pronounced than those of nucleic acid. By this theory, chromatin is ordinarily the histone salt of histone-nucleic acid. There does not seem, however, to be any convincing evidence that these two bondings may not both be essentially salt-like.

Observations on the protein component of plant nuclei are very few. *A priori*, it should be a protein with an iso-electric point more basic than the pH of the nuclear fluids, in order to let it function as a base. But as the interior pH of most plant cells is fairly acidic, this prerequisite is fulfilled by a considerable range of possible proteins. In the embryo of the garden pea (45) analyzed without separating cytoplasm from nuclei, a nucleoprotein fraction was extracted in which, combined with the thymonucleic acid, were two proteins, of which the first was water-soluble and rich in tyrosine, while the other was alkali-soluble and rich in dicarboxylic amino-acids. It is to be presumed that at least one of these, but not necessarily both, was derived from the nucleus. Nothing was found that would be comparable to protamine or histone, and the proteins found did not show a basic reaction.

A recent chapter has been added (6, 7, 8) to the history of the organic analysis of nuclei through the invention of new methods of obtaining the separated nuclei. Behrens has shown that after grinding tissues to an ultra-fine powder it is possible to sort the cell components according to their specific gravity by centrifugation, and that usually the chromatin is separable as the heaviest component. By this method it was verified that the nuclei of rye germ (27) contain thymonucleic acid and not the so-called vegetable nucleic acid—the latter being, in fact, a cytoplasmic constituent. The application of Behrens' technic has also led to the re-discovery of sulphur-rich material in the nucleus. This observation of sulphur, first recorded by Miescher (81) in the nuclei of human pus and again the heads of bull sperm, is now reported in considerable

abundance by D. T. Mayer (see 35) in calf thymus nuclei. The significance of such sulphur-rich protein and the range of its occurrence have not yet been determined

The Behrens' technic (7) gives a product that can be examined for enzymes, and he reports that thus far he has found arginase associated with both nucleus (chromatin?) and cytoplasm, but has found that cells possessing catalase, lipase and amylase show them only in the cytoplasm. A report by Bunding (10), using a different method, confirms the limitation of catalase to the cytoplasm. As for other enzymes, the earlier opinion that the nucleus is a reservoir of respiratory oxidases remains unproved and unrefuted (121, 122). The influence of nuclear events on cell oxidation has been made very clear, but it remains possible that the locus for the enzyme synthesis may be in the cytoplasm.

LIPIDS

Exceedingly little is known about the lipid content, either of chromosomes or of total nuclei. The reports of Miescher and Schmiedeberg (86) indicated a small and quite variable content of ether-soluble material in the salmon sperm-head fraction—0.7% to 2.5% of the dry weight. Mathews' figures (78) from herring sperm head ran still lower—about 0.1%. Burian's review (12) of these studies endorses Schmiedeberg's suggestion that even this small amount may be due to contamination from the lipid-rich tails and that very possibly lipids are foreign to the nuclei in general.

In some of the tissues of higher animals treatment with citric acid will cause the nuclei to slip loose, enclosed in their membranes. Stoneburg (113) analyzed these separated nuclei for lipids, which comprised in his preparations 8% to 10% of the total dry weight of the nuclei. They seem to be structural material rather than metabolic or nutritional. Only about one-fifth was found to be fatty acids and neutral fats, and the remainder was about equally divided between cholesterol and a peculiar phospholipin fraction. The solubilities of the latter differed from that of the usual unsaturated lecithins and cephalins, and pointed to either sphyngomyelin or a saturated lecithin or cephalin. The high relative proportion of cholesterol in their mixutre is conspicuous. There is no evidence what part of these lipids was contained within the nuclear structures and what part may belong to the outer membrane.

Thus, although the condensed chromatin of the spermatozoa is nearly (or quite?) free of lipids, it appears that some varieties of metabolizing nuclei possess a very interesting ether-soluble fraction.

It is problematical whether the so-called nucleus of the yeasts and of some other lowly plants ought to be considered here. The "nuclear" stain which these give has been ascribed not to nucleic acid compounds but to an acidic lipoprotein. The Gramm technic has been explained (106) as the hydrolytic release of the fatty component, followed by its selective staining with gentian violet. It is claimed that any basic dye of the fuchsin series will give this effect. But in contradiction to all this, more recent reports claim that the yeast "nucleus" contains a Feulgen-positive, thymine-containing nucleic acid (20).

MINERAL CONTENT

The earliest study of the mineral content of nuclei was Miescher's examination (86) of the ash of salmon sperm heads. He found that the residue, heated to incandescence, contained from 1.56% to 1.83% of the fat-free dry weight. Much, or perhaps even all of this, may represent organically combined material, or the sparingly ionizable salts of inorganic bases with biological acids.

Phosphorus is an important element in chromosomes, since it is a necessary part of the nucleic acid molecules. Stoneburg's researches (113) point to its occurrence also as phospholipin in the nucleus, but do not determine whether this form is located in the chromosomes.

Chlorides, ionized or otherwise, have never been shown present in nuclei, so far as the available literature discloses. Macallum (73, 75) looked for them in vain. Inasmuch as he found them in cytoplasm, both present and difficult to wash away, the negative finding in nuclei may have genuine significance. But it is necessary to speak cautiously when discussing such a mobile ion.

Sodium is a highly doubtful element. Being very nearly absent from all parts of land and fresh water plants, and in animals chiefly characteristic of the intercellular fluids rather than the cellular structures, we are left with no particular reason for expecting it in the nucleus, except for the difficulty in supposing a mechanism that could completely exclude it. Macallum (75) argues that it is absent because nuclei seem to be devoid of chlorides.

Potassium, an almost universal element in the cytoplasm of cells, deserves to be considered seriously as a possible cation in chromosomes. Macallum (74, 75) conducted extensive explorations of the apparent distribution of potassium, using as a test the formation of insoluble potassium cobaltic hexanitrite. Potassium appears by Macallum's investigation to be universally absent from nuclei, but it must not be forgotten that the reagent has a lower diffusibility through membranes than the ion to be tested. For this reason, Lison (66) doubts that any conclusion can be drawn from Macallum's observations. Lison's doubts seem particularly well founded for plants, as Macallum's plant-cell preparations frequently show the cobalt precipitates in close contact with the outer surface of nuclear membranes. Among animal cells it is significant that Macallum (74) found certain forms (*e.g.*, ganglion cells of higher animals) in which neither nucleus nor cytoplasm reacted for potassium. As any supply existing in the nucleus could hardly have diffused out completely to the exterior of the cells, it may be judged that these nuclei were potassium-free, and that the element is at least not always necessary to the nucleus.

Miescher and Schmiedeberg (86; see also 102) found in their nuclear ash easily soluble calcium to the extent of .23% of the oven-dry substance. They surmise that most of the rest of the ash is calcium sulphate, making the hypothetical calcium content come to about 0.6%. Microincineration tests by Policard and his school (92–96) show that chromosomes and nucleoli leave a copious ash having the character of the oxides (and phosphates?) of the alkaline earths. G. H. Scott (107, 108) uses the electron microscope to confirm that the cations in the ash must be calcium, magnesium, or a mixture of the two. This ash is closely associated with the formed structures in the nucleus, and in the chromosomes (4) the points of concentration correspond to those of nucleic acid. There are abundant inferential reasons for expecting that on further investigation these elements will be found to exist here as relatively low-ionizing combinations with the organic constituents.

Positive microchemical tests for iron were reported by Macallum (69, 71, 72, 75) in 1891 in the chromatin of all nuclei. He believed that chromatin was very closely related to hemoglobin (70) and, indeed, that it was the mother substance for the hemoglobin of vertebrate blood cells. Miescher in 1892 (84) found iron in prepa-

rations of salmon spermatozoa that had undergone long storage. He thought at that time that the element was part of an organic compound of high molecular weight existing alongside the protamine and the nucleic acid (both of which he reported to be iron-free) and that this was the substance responsible for the principal staining properties of nuclei. Hence, he coined the designation "karyogen." But Miescher's final works, as edited after his death by Schmiedeberg (86), eliminated "karyogen" or any other explicitly iron-containing compound from the salmon-sperm picture. Burian argued in 1906 (12) that "karyogen" should be recognized as a nuclear component, not present in great quantity, but, he believed, extremely rich in iron. Others at various times (*e.g.*, 39, 99) have followed in the footsteps of these authors. Gilson, 1892 (29), was the first to voice the warning that, since nucleic acid has a most intense affinity for iron and since the quantity of iron necessary for a positive test is fabulously small, there must be a serious suspicion that histochemically detected iron may easily come from external sources. (See also 68, 114.)

All the investigators agree that no ferric or ferrous ions are present at the start. In order to test for "masked," or organically bound iron, some chemical treatment is needed, either acid to hydrolyze the supposed bond, or, according to Macallum, prolonged treatment with ammonium sulphite, which can be carried out on a microscope slide in presence of dilute glycerol. If the "unmasking" is done with acid, hematoxylin or ammonium sulphide are afterwards needed to develop the iron picture. The weak spot in the test is that even alkaline ammonium sulphide is quite good enough a solvent to transport into the nuclei any iron that may be present, as a contamination or otherwise, in any part of the preparation. The present author has verified (33) that if slide preparations are handled with ammonium sulphide under extreme precautions against contamination, the nuclei will generally stand for weeks without showing a reaction for iron. If a minimal trace of iron is then added to the edge of the preparation, in a few days every nucleus will be strongly tinged with the dark olive of FeS. The actual quantity of iron needing to enter a nucleus in order to give its chromosomes a strong reaction is certainly less than 1×10^{-9} milligram. It appears, then, that a considerable share of the Macallum iron reactions in chromatin are quite probably due to outside contaminations—

doubtless in many cases only fantastically small—and for the remainder it is impossible to know the pre-mortem location of the iron, since the chromosomes are sure to take it over whenever it is released.

Micro-incinerations, carried out by Policard and associates (95, 96), show sperm heads and nuclei with the colorless ash of the alkaline earths or their phosphates, not detectably discolored with iron oxide.

None of the above investigations shuts out the possibility of iron-containing active principles, such as the enzymes, since these would require less of the element than it would take to give a colored ash, and the technics used for "unmasking" the element are seldom vigorous enough to liberate it from its position in a possible porphyrin combination. The most important of such enzymes are catalase and certain peroxidases. We have already mentioned that in animal tissue catalase (7, 10) is found to be a cytoplasmic rather than nuclear enzyme. The distribution of peroxidases in the cell has not as yet been determined.

ORGANIC MICRO-CHEMICAL TESTS

Very few really microchemical methods for detecting organic materials are applicable to chromosomes. One of the most important is the "nucleal" stain devised by Feulgen (28, 115, 123), serving to locate and distinguish thymonucleic acid. When killed and fixed nuclei are subjected to very gentle hydrolysis the thymonucleic acid in them loses its purine bases, liberating the aldehyde groups on two of the desoxyriboses present. This makes possible a histo-chemical variant of Schiff's color reaction between aldehydes and a colorless solution of fuchsin-sulphurous acid. If pre-existent aldehydes and alcohol-soluble lipoid aldehydes have been eliminated, the reaction is found to be very specific for thymonucleic acid, *i.e.*, for desoxyribonucleic acid. This reaction is responsbile for the first discovery that the nucleic acid in the nuclei of numerous higher plants agrees rather with animal nuclei than with their own previously discovered (cytoplasmic) ribonucleic acid. The latter acid gives no Feulgen reaction, apparently because it is resistant to the degree of hydrolysis specified for the test. A long list of Feulgen tests made by Boas and Biechele (9) shows that there are many plant nuclei that give a negative reaction, as well as those that are positive. There are

no indications what the chromosome nucleic acids may be in the plants that react Feulgen-negative.

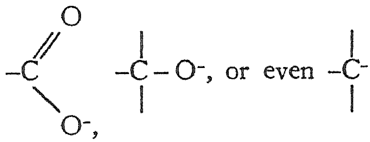
Caspersson (14, 15; see also 127) has carired out a notable series of investigations on the cytological distribution of nucleic acid, making use of some novel methods. Depending on the fact that all molecules containing a pyrimidine group show a powerful absorption band at approximately the wave length, 2600 Å, and that at this particular zone other biological materials show only moderate absorption, he was able to photograph the location of nucleic acid in both living and fixed nuclei by the use of monochromatic ultra-violet light of this wave length. In the giant chromosomes from the salivary cells of diptera he could show that the distribution of nucleic acid corresponds precisely to the bands of intense staining with the usual nuclear dyes. For a confirmatory test he digested the nuclei with trypsin in the presence of lanthanum salts, dissolving away all the nuclear structures except the nucleic acid which was left in place as its insoluble lanthanum compound. At appropriate stages in this digestion he could demonstrate the subdivision of the chromatin into even shorter units (down to 0.1 μ) than had been visible by other methods. But with completed digestion the deproteinated chromosomes were left as discontinuous fragments, evidently robbed of their organizing framework.

A mode of digestion, the converse of that by Caspersson, was carried out by Mazia and Jaeger (79, 80). This consisted in digesting out the nucleic acid with a tetranucleotidase, leaving these chromosomes in a sense still entire, but stripped of their differential banding. By comparing these two digestion experiments we are led to conclude that the framework of chromosomes is built of protein digestible by trypsin, and that the nucleic acid is built into this framework in special locations.

THE INTERPRETATION OF STAINING REACTIONS

Histological stains are of many kinds, based on many different underlying principles. A few differential stains seem to depend upon unexplained special affinities. Some exemplify processes of selective solid solution. Much the greatest number of stains behave, however, in accordance with the rules of salt formation, as modified by the peculiar state of aggregation of the substances concerned. To explain this, it will be necessary to give a little attention to the mode of ionization of amphoteric solids in contact with water.

With the exception of certain fatty substances, nearly all the biological carbon compounds are able to release hydrogen or hydroxyl ions when in contact with water. If the compound contains no nitrogen, the released ions are almost always hydrogen. and the compound thereby shows at least a vestige of acidic character, strong or weak in proportion to the degree of the tendency to ionize. Balancing the electropositive charges of the hydrogen, the organic molecules are left with an electro-negative charge, or if we are dealing with a solid, the surface in contact with water is sprinkled with electro-negative ionic groupings, such as:—



Organic substances containing oxidised sulphur or phosphorus have this acidic property much intensified. If the compound contains nitrogen, especially $-NH_2$ groups, the nitrogen is prone to add the elements of water and thereupon to ionize off a negatively charged hydroxyl. Nitrogenous compounds tend thereby to be left with an electropositive charge, so that they behave as bases, or their water-contact areas as basically reacting surfaces. Such surfaces have the power to make loose, salt-like combinations with dissolved ions of the opposite sign. The soft, water-filled solids of the plant or animal cell may be considered to have this surface of water contact extended inward through the whole mass, and consequently the power to ionize and to build salt compounds extends through the thickness of these semi-solid structures. By taking the color ion of a dye substance to be the water-soluble ion in this reaction, we arrive at the "chemical" theory (91, 111, 112) of histological staining, synonymous today with the "electrical" theory (5), although these were earlier looked upon as contrasted theories.

The majority of histological structures is built of molecules that carry both basic nitrogen and ionizable hydrogen on different positions of the same molecule. But the physical constants for these two types of dissociation are such that an appreciable liberation of either H or OH ions is possible only when the other of these two ions is effectively held in its place upon the solid. An electronegative or acidic surface will be generated through release of H ions only

when the aqueous phase lacks the excess of H ions, the mass effect of which would suppress the ionization of the surface. Conversely, a relative shortage of OH ions in the aqueous solution is prerequisite to the release of OH ions by the solid. In short, acidity in the watery medium helps the solid surface to behave as a base, and *vice versa.*

In a majority of cytological structures the acidic characteristics over-balance the basic in the same molecules. Hence, the iso-electric point, or point at which the two ionizations are suppressed in equal measure, lies on the acid side of neutral. This is recorded in terms of the pH of the aqueous solution that brings about such a balance—it being remembered that pH 7 represents neutrality, the pH figures above or below 7 running inversely to the hydrogen ion concentration or acidity. The iso-electric points (40, 128, 129, 130) of cytoplasmic structures vary over a wide range, with the majority of cases between 4 and 6, but a few, such as mucin (mucoitin-sulphuric acid) and phosphoprotein (partially esterified phosphoric acid), running to substantially more acid figures. The chromatin iso-electric point in the nuclei is reached only at pH values of 3.5 to 2.8. Incidentally, these figures must not be confused with the actual pH of the cell fluids (116, 117, 98) which have been shown in certain instances to be less acid in nuclei than in cytoplasm.

Applying these principles to cytological staining, a basic dye in a neutral medium, or a dye mordanted by a base in a neutral solution, will stain almost all cell structures, because they are built mostly of materials that are at least to some degree acidic. But if the solutions are set at a pH between 3 and 4 by the use of free acids or acid salts (like the alums), nearly every structural element except the nucleic acid will cease to show acid characteristics, and staining with a basic dye will give good differentiation of the chromatin. Under these conditions the ordinary proteins (iso-electric point 4.7 to 6) will have the staining properties of bases and be classed "acidophilic." If nucleic acid is to be differentiated from materials, such as mucin and phosphoprotein, carefully adjusted acid fluids are necessary, to come below their iso-electric points without running below the iso-electric point of the chromatin.

In order to produce chemically interpretable results, it is necessary to stain by the "progressive" rather than the "regressive"

methods, or else to use such slow procedures that a chemical equilibrium is reached. In the "regressive" stains, differentiation is obtained by indiscriminate staining followed by washing out with acid or some other solvent. Usually, there is no attempt to come to chemical equilibrium, so that all that is demonstrated is a difference in the speed with which the color is removed from different structures, and this speed is a function controlled by many variables.

One should keep in mind that although chromosomes are characterized by the strongest of the histological acids, it is there in combination with the most strongly basic proteins of the cell. Under right conditions these basic proteins may exert a determining influence upon the histological stain. The histological distinction between basichromatin and oxychromatin (14) is founded upon the different organic stains taken by different parts of the nucleus, when handled at approximately pH 4.6. At this arbitrary pH most chromatin structures are still "basophil" ("basichromatin") because still predominantly under the influence of the nucleic acid content. But certain parts of some nuclei placed at this pH will, instead, select the acidic organic dyes ("oxychromatin") because their wealth of highly basic proteins will raise the total iso-electric point sufficiently toward neutral to make it lie above the pH of the staining solutions. By comparing with the results of ultraviolet photography, Caspersson showed that these differences in staining were not correlated with any systematic difference in content of nucleic acid, the shadows of which are seen in his photographs in both sorts of chromatin.

It seems that the crucial iso-electric point for the staining of a particular structure varies according to the procedure that is being employed. Basic "aniline" dyes used in "progressive" staining often cease to color even "basichromatin" (67) when brought to pH 3, the approximate iso-electric point, apparently, of one of the protein-nucleic acid compounds present. But if stained "progressively" with the aid of a heavy metallic mordant the effective iso-electric point of chromatin (19) may run lower than pH 1.2, indicating a relation of mordant to tissue governed by the iso-electric point of liberated nucleic acid. Such a stain may count as a definite proof of nucleic acid, since at least in the nucleus a second acid of that strength is out of the question. A more systematic use of these progressive stains in conjunction with solutions buffered to

different pre-determined acidities should lead us to a greatly extended knowledge of the ionic characteristics of the cell parts.

ITEMS IN DIMENSIONAL CHEMISTRY

Certain fundamental dimensional units have a bearing on our pictures of the finer organization of chromosomes. The polypeptide chains of protamines, when extracted and studied by themselves, run in a fine zigzag, the angles of which represent, in case there are no stresses, the characteristic $109\frac{1}{2}°$ angular deviation between the valencies of the carbon atom. The average linear distance between atoms (18) represented by successive points in the zigzag is about 1.43 Ångstrøm units (10,000 Å = 1 μ). As indicated in Fig. 2, every third atom in the series provides the point of attachment of an amino-acid residue as a side-chain, designated as "R." Dotted lines running through the diagram divide out the successive amino-acid residues. The space taken up by each of these (1) amounts to 3.5 Å, as determined by x-ray.

The protamine molecule (65, 119) in fish spermatozoa is a chain molecule carrying from 15 to 30 amino-acids in serial order. These protamines are a sort of last-minute substitution for the histones in the chromosomes of the unripe cells, and if we are not to suppose an incredibly radical reconstitution of the chromosomes during the ripening of the sperm, we must presume that the histones that were previously present had a similar filamentous polypeptide pattern, in spite of a molecular size representing perhaps eight times the content of total amino-acids.

The molecular structure here described corresponds to that of the mechanically strong and not very extensible protein fibers, such as silk. But when living chromosomes are subjected to micro-manipulation, they are found to have long-range elasticity (17, 77), more like rubber or like the protein fibers of elastic tendon materials for which a highly serpentine type of molecular strands must be assumed. It waits to be learned what orientation and what system of looping would be consistent with known chromosomal structures. The scale of this folding must be easily a hundred times smaller than the structures seen near the limits of vision in the "bottle-brush" chromosomes (13) of some authors. While the main direction of the molecular fibers presumably would be longitudinal to the chromosome, it must be adequate in the several di-

rections of space to supply a chromatin-free chromosome with its three-dimensional cohesion. The protein chain must be supposed to terminate at one end in a carboxyl group, but in the case of pro-

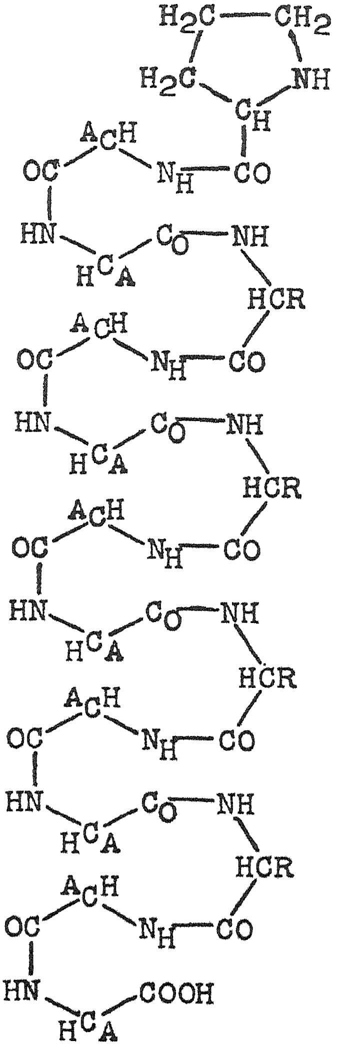


FIG. 3. Plausible serpentine form for natural condition of protamine in fish sperm nuclei. A = arginine (basic) residues. R = amino-acid residues other than arginine and proline. A proline ring is represented as occupying a terminal (top) position.

tamines the reverse end does not culminate in the expected $-NH_2$ (120) group, as the position of the terminal amino-acid is occupied by proline, a variant of the amino-acid pattern carrying no free $-NH_2$. This precludes the end-to-end salt-like union of chains that has sometimes been postulated. By analogy with other biological materials, the most natural supposition would be that these extended molecular strands run lengthwise of the chromosomes, but real evidence for or against this orientation is not yet in hand. In any case, the geography of the chromosomes is established by the protein component more than by nucleic acid.

The minimal molecule of thymonucleic acid (1, 2, 3, 25, 46, 61, 101, 109) is the tetranucleotide, molecular weight 1254, but the freshly extracted substance tends to appear in solutions in a micellar or polymerized condition with a molecular weight varying from 500,000 to over 1,000,000, averaging perhaps near to 600,000, roughly equivalent to 500 of the minimum molecules or 2,000 nucleotide units. The resulting "micelle" or super-molecule is long and slender, in the ratio Length: width 300: 1. None of the acidic hydrogens is buried in the interior of the micelle. Polarized light (103) reveals a predominantly transverse structure, and x-ray diffraction indicates that the spacing of this structure is on a rhythm of 3.34 Å. These data present us with a super-molecule 6,000 to 7,000 Å (0.7 μ) in length and about $15 \times 7\frac{1}{2}$ Å in cross section, consisting of a series of mononucleotides lying transversely and each taking up 3.34 Å of the length of the super-molecule. Evidently, there is a remarkably close packing of the nucleotide units—like pennies in a bank clerk's roll—indicative of a compact flat configuration of the cyclic components of the nucleotides. This may weigh to some extent as an argument that the desoxyriboside ring has the pentagonal configuration, as favored by Levene, (64) rather than the hexagonal, since the geometry of the carbon bonds allows the pentagon to lie more successfully in a flat plane. In that case, the phosphorus bridges from one nucleotide to the next may well be visualized as loops in a sort of cork-screw spiral (Fig. 1.)

On the basis of their polariscopic character (44, 103), chromosomes carry their nucleic acid in molecular strands running lengthwise of the chromonemata. The quantity present (15, 16, 37, 38, 36) fluctuates quite surprisingly during the life history of the cells, so that it is impossible to postulate a nucleoprotein with a clearly

defined percentage of nucleic acid. It is at a minimum in non-mitotic nuclei (14, 16, 105), and in gland cells particularly in cells that have been fatigued by secretion. Rest restores the supply, and during mitosis it rises to a maximum. In the ripe sperm of salmon the condensed chromosomes are as much as 60% of their weight nucleic acid.

A calculation of the nucleic acid content of these salmon sperm nuclei in comparison with the basic combining power of the proteins on hand leads to interesting results. Ninety-six per cent of the dry substance is here protamine nucleate (86, 12). The protamine is now believed to owe its basicity entirely to its arginine content. On this basis, allowing one replaceable H for each P of the nucleic acid, the 14 arginines reported in each protamine molecule supply enough base to satisfy $\frac{14}{16}$ of all the replaceable H. The supply of earthy metal found in the ash of these cells seems sufficient to take care of the remaining acidity. In the case of thymus nuclei, the histone must be very nearly sufficient to satisfy the nucleic acid bonds, but the picture is complicated by the presence of other proteins that are not well known, and it is not possible to judge in what form the ash constituents are held.

THE QUESTION OF THE GENES

In considering the genes, we face the difficulty that for the analytical chemist they virtually do not exist. All indications are against the identification of any major substance in the chromosomes as gene substance (84, letter #78; 12, 58, 33). Nucleic acid is too uniform from species to species. Protamine, the known protein of fish sperm nuclei, is too monotonous in structure and seems not to exist in the cells of the immature germinal epithelium, cells from which the spermatozoa must receive their genes. And histone, the major protein of the testicular cells, is not carried over to the spermatozoa in salmon or herring in quantity sufficient to figure in an analysis. The thought that chromosome substance, in the broad, may be counted as all gene substance, is negated when we consider how impossible such a continuous substrate would make it for mutations to follow the well known discontinuous pattern rather than a smoothly sliding scale of quantitative variation. In the minds of most geneticists today, what we may call the molecular character of Mendelian heredity is most readily explained by sup-

posing that the genes which cause it are themselves actual single chemical molecules—or super-molecules—(21, 33; but contrast 31) having a predetermined molecular size and a definite unit potency in the production of their appropriate biologically active principles.

As long as known substances of the chromosomes are not to be identified with genes, the question of the nature of the gene substance can not quite escape from the cloudland of things speculative. The gene super-molecule must undoubtedly have the character of an enzyme (30, 47, 32, 33, 34), in the first place as a substance catalysing the synthesis of its own duplicate, and secondly as an enzyme producing some active principle. Since enzyme characteristics are, so far as we know, limited to protein substances, we naturally suppose that each gene is a protein. The close correlation that is thought to exist between the power of enzymatic self-reduplication and the presence of nucleic acid, (2, 106, 110, 118) especially as we see it in the virus proteins, leads to the thought that genes may be nucleoprotein combinations, an idea all the more plausible because it gives them a nature harmonious with their surroundings.

The process of self-reduplication implies the possibility that a pre-existing molecule can serve as a pattern upon which the new can be built, in much the same way that the atomic configuration on the surface of a growing crystal can serve as a master pattern to control the comparable placement of new tiers of crystal (32). For such a mechanism to succeed, it is necessary that the fundamental unit of gene substance should be virtually without thickness, in short that it should be a linear molecule or a thin mono-layer, not a "globular" protein. Or at the furthest, one might consider the difficult possibility of a box-like structure (34, 126) with mono-layer walls, provided that the box unfolds into a flat form when reduplicating, or that it has such a shape that the spacings between boxes are the same shape as the boxes themselves, thus furnishing an appropriate location in which the duplicate molecules can be built. Among these alternatives the most attractive hypothesis is probably some sort of very precisely constituted protein mono-layer, because of the easy adaptability of such a pattern to the requirements, while allowing at the same time for almost any degree of complexity. When once we can verify the molecular pattern of an uninjured native protein mono-layer, much light will be thrown on the possible configuration of the gene substance. But as yet, these attempts to imagine

their chemical constitution do little more than exemplify the necessary striving of science to encompass the hitherto unknown.

BIBLIOGRAPHY

1. ASTBURY, W. T. 1939. The x-ray study of protein and related structures. Sci. Prog. 34: 1–19.
2. ————. 1939. Protein and virus studies in relation to the problem of the gene. Proc. 7th Int. Genet. Congr., p. 49–51.
3. ————, AND BELL, FLORENCE O. 1938. X-ray study of thymonucleic acid. Nature 141: 747–748.
4. BARIGOZZI, C. 1938. La signification du spodogramme pour l'étude de la structure des chromosomes. Bull. Hist. Appl. Physiol. 15: 213–219.
5. BAYLISS, W. M. 1906. On some aspects of adsorption phenomena with especial reference to the action of electrolytes and to the ash constituents of protein. Biochem. Jour. 1: 175–229.
6. BEHRENS, MARTIN. 1932. Untersuchungen an isolierten Zell- und Gewebsbestandteilen. I. Mitteilung: Isolierung von Zellkernen des Kalbsherzmuskels. Ztsch. Physiol. Chem. 209: 59–74.
7. ————. 1935. Mechanische Isolierung von Zell- und Gewebsbestandteilen in grösseren Mengen. Verh. Deut. Path. Ges. 28: 157–158.
8. ————. 1938. Zell- und Gewebetrennung. Abd. Hanb. Biol. Arbeitsmethoden, Abt. V, Teil 10, pp. 1363–1392.
9. BOAS, F., AND BIECHELE. O. 1932. Über die Feulgensche Nuclealreaction bei Pflanzen. Biochem. Ztsch. 254: 467–474.
10. BUNDING, IRBY M. 1941. The location of catalase in the cell. Jour. Cell. & Comp. Physiol. 17: 133.
11. BURIAN, RICHARD. 1904. Chemie der Spermatozoen. I. Ergeb. Physiol. 3: 48–106.
12. ————. 1906. Chemie der Spermatozoen. II. Ergeb. Physiol. 5: 768–846.
13. CALVIN, M., KODANI, M., AND GOLDSCHMIDT, R. 1940. Effects of certain chemical treatments on the morphology of salivary gland chromosomes and their interpretation. Proc. Nat. Acad. Sci. 26: 340–349.
14. CASPERSSON, TORBJÖRN. 1936. Über den chemischen Aufbau der Strukturen des Zellkernes. Skan. Arch. Physiol. 73: Suppl. No. 8 (151 pp.). [See also 1937, same title Protoplasma 27: 463–467.]
15. ————. 1939. On the role of the nucleic acids in the cell. Proc. 7th Int. Genet. Congr. p. 85–86.
16. ————, AND SCHULTZ, JACK. 1938. Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Nature 142: 294–295.
17. CHAMBERS, ROBERT. 1924. The physical structure of protoplasm as determined by microdissection and injection. General Cytology (E. V. Cowdry) U. of Chicago Press (See pp. 265–276).
18. COREY, R. B. 1940. Inter-atomic distances in proteins and related substances. Chem. Rev. 26: 227–236.
19. CRAIG, RODERICK, AND WILSON, CHARLES. 1937. The use of buffered solutions in staining; theory and practice. Stain Tech. 12: 99–109.
20. DELAPORTE, BERTHE, AND ROUKHELMAN, NADIE. 1938. Sur la présence de la thymine dans l'acide nucléique extrait de la levure. Recherches cytologiques et chimiques. Comp. Rend. Soc. Chim. 206: 1399–1401.
21. DEMEREC, M. 1933. What is a gene? Jour. Heredity 24: 369–378.

22. DIETERLE, R. R. 1930. A modification of Macallum's hematoxylin method for iron. Arch. Path. 10: 740–741.
23. FELIX, K., AND RAUCH, H. 1931. Über den Aufbau des Histons der Thymusdrüse. Ztsch. Physiol. Chem. 200: 27–40.
24. FEULGEN. R. 1927. Über die für die Nuclealreaction und Nuclealfärbung verantwortlich zu machenden Gruppen. Ztsch. Physiol. Chem. 165: 215–223.
25. ————. 1935. Über a- und b-Thymonucleinsäure und das die a-Form in die b-Form überfuhrende Ferment (Nucleogelase). Ztsch. Physiol. Chem. 237: 261–267.
26. ————. 1935. Histochemischer Nachweis von Aldehyden. Verh. Deut. Path. Ges. 28: 159–161.
27. ————, BEHRENS, M., AND MAHDIHASSAN, S. 1937. Darstellung und Identifizierung der in den pflanzlichen Zellkernen vorkommenden Nucleinsäure. Ztsch. Physiol. Chem. 246: 203–211.
28. ————, AND ROSSENBECK, H. 1924. Mikroskopischchemischer Nachweis einer Nucleinsäure. Ztsch. Physiol. Chem. 135: 203–248.
29. GILSON, G. 1892. On the affinity of nuclein for iron and other substances. Rep. Brit. Assn. Adv. Sci. 1892: 778–780.
30. GOLDSCHMIDT, RICHARD. 1927. Physiologische Theorie der Vererbung. 247 pp.
31. ————. 1938. The theory of the gene. Sci. Monthly 46: 268–273.
32. GULICK, ADDISON. 1937. Some contributions of chemistry to the theory of the gene. Proc. Mo. Acad. Sci. 3: 75–77.
33. ————. 1938. What are the genes? II. The physico-chemical picture; conclusions. Quart. Rev. Biol. 13: 140–168.
34. ————. 1939. Growth and reproduction as problems in the synthesis of protein molecules. Growth 3: 241–260.
35. ————. 1939. Analysis of nuclear material obtained by differential centrifugation of finely powdered glandular tissue. Proc. 7th Int. Gen. Congr. p. 135.
36. HAHN, L., AND HEVESAG, G. 1940. Turn over rate of nucleic acid. Nature 145: 549–550.
37. HAMMARSTEN, EINAR. 1924. Zur Kenntnis der biologischen Bedeutung der Nucleinsäureverbindungen. Biochem. Ztsch. 144: 383–466.
38. ————. 1939. The function of thymonucleic acid in living cells. Jour. Mount Sinai Hosp. 6: 115–125.
39. HAMMARSTEN, OTTO. 1894. Zur Kenntnis der Nukleoproteide. Ztsch. Physiol. Chem. 19: 19–37.
40. IKEDA, SIZUE. 1936. Zusammenhang zwischen den isoelektrischen Punkt und einigen mikrochemischen Reaktionen beim Augapfel des Herbstfrosches. Folia Anat. Japon. 14: 175–180.
41. JANSSEN, L. W. 1939. Biosynthesis and the outlines of protein structures. Protoplasma 33: 410–426.
42. JONES, WALTER. 1920. The chemical composition of adenine nucleotide and of yeast nucleic acid. Amer. Jour. Physiol. 52: 193–202.
43. JORDAN-LLOYD, D. 1937. Recent development in our knowledge of the protein molecules. *In*: Perspectives in Biochemistry (Needham and Green, Editors), p. 23–35.
44. KIESEL, A. 1930. Chemie des Protoplasmas. 302 pp.
45. ————, AND BELOZERSKI, A. 1934. Über die Nucleinsäure und die Nucleoproteide der Erbsenkeime. Ztsch. Physiol. Chem. 229: 160–166.
46. KLEIN, V., AND ROSSI, A. 1935. Experimentelle Studien über den Nukleinstoffwechsel. XXXVI. Fermentative Untersuchungen über den Aufbau des Polynucleotids. Ztsch. Physiol. Chem. 231: 104–114.
47. KOLTZOFF, N. K. 1928. Physikalisch-chemische Grundlage der Morphologie. Biol. Ztrbl. 48: 345–374.

48. Kossel, Albrecht. 1879. Ueber das Nuclein der Hefe. Ztsch. Physiol. Chem. **3**: 284–291.
49. ———. 1882. Zur Chemie des Zellkerns. Ztsch. Physiol. Chem. **7**: 7–22.
50. ———. 1884. Ueber einen peptonartigen Bestandteil des Zellkerns. Ztsch. Physiol. Chem. **8**: 511–515.
51. ———. 1886. Weitere Beiträge zu Chemie des Zellkerns. Ztsch. Physiol. Chem. **10**: 248–264.
52. ———. 1891. Über die chemische Zusammensetzung der Zelle. Arch. Anat. Physiol. **1891**: 181–186.
53. ———. 1897. Ueber die basischen Stoffe des Zellkerns. Ztsch. Physiol. Chem. **22**: 176–187.
54. ———. 1903. Zur Kenntnis des Salmins. Ztsch. Physiol. Chem. **40**: 311–315.
55. ———. 1911. Über die chemische Beschaffenheit des Zellkerns. Münch. Med. Wochensch. **58**: 65–69.
56. ———. 1928. The protamines and histones. 107 pp.
57. Kossel, A., and Kutscher, F. 1900. Beiträge zur Kenntnis der Eiweisskörper. Ztsch. Physiol. Chem. **31**: 165–214.
58. ———, and Schenck, E. G. 1927. Untersuchungen über die basischen Eiweiszstoffe, ein Beitrag zu ihrer Entwicklungsgeschichte. Ztsch. Physiol. Chem. **173**: 278–308.
59. Leipert, Th., and Kurokawa, T. 1933. Zur Chemie der Vogelblutkerne. Biochem. Ztsch. **261**: 425–432.
60. Levene, P. A. 1921. On the structure of thymus nucleic acid. Jour. Biol. Chem. **48**: 119–125.
61. ———. 1938. Structure of desoxyribonucleic acid. On the diphosphoric esters of pyrimidine-desoxyribosides. Jour. Biol. Chem. **126**: 63–66.
62. ———, and Bass, L. W. 1931. Nucleic acids. 327 pp.
63. ———, Mikeska, L. A., and Mori, T. 1930. On the carbohydrate of thymonucleic acid. Jour. Biol. Chem. **85**: 785–787.
64. ———, and Tipson, R. S. 1935. The ring structure of thymidine. Jour. Biol. Chem. **109**: 623–630.
65. Linderstrøm-Lang, K. 1935. Some electrochemical properties of a simple protein. Trans. Faraday Soc. **31**: 324–335.
66. Lison, Lucien. 1936. Histochimie animale; méthodes et problèmes. 320 pp.
67. ———, and Fantrez, J. 1939. L'étude physicochimique des colorants dans ses applications biologiques—Étude critique. Protoplasma **33**: 116–157.
68. Lynch, Vernon. 1920. Chemistry of the whitefish sperm. Jour. Biol. Chem. **44**: 319–328.
69. Macallum, A. B. 1891. On the demonstration of the presence of iron in chromatin by micro-chemical methods. Proc. Roy. Soc. (London) **50**: 277–286.
70. ———. 1893. Studies on the blood of amphibia. Trans. Can. Inst. **2**: 221–260.
71. ———. 1895. On the distribution of assimilated iron compounds, other than hemoglobin and hematins, in animal and vegetable cells. Quart. Jour. Micr. Soc. **38**: 175–274.
72. ———. 1897. On a new method of distinguishing between organic and inorganic compounds of iron. Jour. Physiol. **22**: 92–98.
73. ———. 1905. On the nature of the silver reaction in animal and vegetable tissues. Proc. Roy. Soc. (London) B **76**: 217–229.
74. ———. 1905. On the distribution of potassium in animal and vegetable cells. Jour. Physiol. **32**: 95–128.
75. ———. 1908. Die Methoden und Ergebnisse der Microchemie in

der biologischen Forschung. II. Das Eisen in Geweben und Zellen. Ergeb. Physiol. **7**: 565–600.

76. ———. 1925. Purpurin method of locating calcium. Science **62**: 511.

77. Manton, Irene. 1939. Evidence on chromosome structure in *Osmunda*. Proc. 7th Int. Gen. Congr., p. 210.

78. Mathews, A. P. 1897. Zur Chemie der Spermatozoen. Ztsch. Physiol. Chem. **23**: 399–411.

79 Mazia, D., and Jaeger, Lucina. 1939. Nuclease action, protease action and histochemical tests on salivary chromosomes of Drosophila. Proc. Nat. Acad. Sci. **25**: 456–461.

80. ———, and ———. 1939. Nuclease action, protease action, and histochemical tests of salivary chromosomes of *Drosophila*. Proc. 7th Int. Genet. Congr. p. 212.

81. Miescher, Friedrich. 1871. Über die chemische Zusammensetzung der Eiterzellen. Med.-chem. Untersuch. Lab. Angew. Chem. Tübingen **4**: 441. (Re-published in 85, 1897, Vol. 2, p. 3–23.)

82. ———. 1874a. Die Spermatozoen einiger Wirbelthiere. Verh. Naturf. Ges. Basel **6**: 138–208. (Re-published in 85, 1897, Vol. 2, p. p. 55–107.)

83. ———. 1874b. Das Protamin, eine neue organische Basis aus den Samenfäden des Rheinlachses. Ber. Deut. Chem. Ges. **7** (I): 376–379.

84. ———. 1897. Die histochemischen und physiologischen Arbeiten. Vol. 1, Wissenschaftlicher Briefwechsel, 138 pp. (For "Karyogen" see letters 72 (p. 110), 73 (p. 110–111), 76 (p. 117–119), 77 (p. 119–122).)

85. ———. 1897. Die histochemischen und physiologischen Arbeiten, Vol. 2, F. C. W. Vogel, 543 pp.

86. Miescher, Friedrich, and Schmiedeberg, O. 1896. Physiologische-chemische Untersuchungen über die Lachsmilch. Arch. Exper. Path. Pharm. **37**: 100–155. (Re-published in 85, p. 359–414.)

87. Milovidov, P. 1938. Bibliographie der Nucleal- und Plasmalreaktionen. Protoplasma **31**: 246–266. (Over 200 entries.)

88. Mühlmann, M. 1928. Hamatoxylin als Reagens auf Eisen. Arch. Pathol. Anat. **266**: 697–711.

89. Neumann, A. 1898. Zur Kenntnis der Nukleinsubstanzen. Arch. Anat. Physiol. **1898**: 374–378.

90. Pavulans, J. 1940. Ueber die Nuclealreaktion in Embryosäcken und Pollenkörnern einiger Angiospermen. Protoplasma **34**: 22–29.

91. Pischinger, A. 1926. Die Lage des isoelektrischen Punktes histologischer Elemente als Ursache ihrer verschiedenen Färbbarkeit. Ztsch. Zellf. Mikr. Anat. **3**: 169–197.

92. Policard, A. 1923. La minéralization des coupes histologiques par calcination et son intérêt comme méthode histochimique général. Comp. Rend. Acad. Sci. **176**: 1012–1014.

93. ———. 1923. Détection histochimique de fer total dans les tissues par la méthode de l'incinération. Comp. Rend. Acad. Sci. **176**: 1187–1189.

94. ———. 1933. Les matières minérales fixes des éléments séminaux au cours de la spermatogénèse. Bull. Hist. App. Physiol. **10**: 313–320.

95. ———. 1934. Sur l'existence de fer dans le noyau cellulaire. Bull. Hist. App. Physiol. **11**: 216–219.

96. ———, and Rojas, P. 1935. Estudio por microincineracion de los globulos rojos en Cichlasoma fascetum (Teleoteos). Rec. Soc. Argent. Biol. **11**: 164. [By abstract, Physiol. Abstracts **20**: 553.]

97. Pratje, Andre. 1920. Die Chemie des Zellkernes. Biol. Centralbl. **40**: 88–112.

98. REISS, P. 1924. Remarques sur le pH intérieur du noyau cellulaire et ses variations expérimentales. Comp. Rend. Acad. Sci. **179**: 641–643.
99. RIECKER, HERMAN H. 1929. Relation of chromatin to hemoglobin and bilirubin. Jour. Exp. Med. **49**: 937–943.
100. SAMPSON, MYRA M. 1925. Conditions of validity of Macallum's microchemical test for calcium. Science **62**: 500–501.
101. SCHMIDT, G., PICKELS, E. G., AND LEVENE, P. A. 1939. Enzymatic dephosphorylization of desoxyribonucleic acids of various degrees of polymerization. Jour. Biol. Chem. **127**: 251–259.
102. SCHMIDT, L. A., AND GREENBERG, DAVID M. 1935. Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev. **15**: 297–434.
103. SCHMIDT, W. J. 1937. Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma. 388 pp.
104. SCHMIEDEBERG, O. 1900. Über die Nukleinsäure aus der Lachsmilch. Arch. Exp. Path. Pharm. **43**: 57–83.
105. SCHULTZ, JACK. 1939. The function of heterochromatin. Proc. 7th Int. Genet. Congr. pp. 257–262.
106. SCHUMACHER, JOSEF. 1925. Zur Chemie der Zellkerne und einiger Nukleinsäure-Eiweiszverbindungen. Chemie der Zelle und Gewebe. **12**: 175–262.
107. SCOTT, GORDON H. 1940. Mineral distribution in some nerve cells and fibers. Proc. Soc. Exp. Biol. & Med. **44**: 397–8.
108. ————. 1940. An electron microscope study of calcium and magnesium in smooth muscle. Proc. Soc. Exp. Biol. & Med. **45**: 30–31.
109. SIGNER, R., CASPERSSON, T., AND HAMMARSTEN. E. 1938. Molecular shape of thymonucleic acid. Nature **141**: 122.
110. STANLEY, W. M. 1939. Properties of viruses. Medicine **18**: 431–442.
111. STERN, ALLEN E., AND STEARN, ESTHER W. 1929. The mechanism of staining explained on a chemical basis. I. The reaction between dyes, proteins and nucleic acid. Stain Tech. **4**: 111–119.
112. STEARN, ESTHER W., AND STEARN, ALLEN E. 1928. The effect of the chemical nature of a decolorizer and its functioning. II. The apparent isoelectric point. Stain Tech. **3**: 87–93.
113. STONEBERG, CHAS. A. 1939. Lipids of the cell nucleus. Jour. Biol. Chem. **129**: 189–196.
114. TAKAHATA, Y. 1924. Über den Eisengehalt der Nucleoproteide. Ztsch. Physiol. Chem. **136**: 214–218.
115. TOMASI, J. A. DE. 1936. Improving the technic of the Feulgen stain. Stain Tech. **11**: 137–144.
116. VLÉS, FRED, AND NOVEL, S. 1922. Notes sur quelques propriétés physico-chimiques des produits sexuels de l'Oursin. Arch. Phys. Biol. **1**: 301–320.
117. ————, REISS. P., AND VELLIENGER, E. 1924. Mesures potentiométriques sur le pH de la substance des oeufs des Oursin. Comp. Rend. Acad. Sci. **179**: 349–351.
118. WADDINGTON, C. H. 1939. The physicochemical nature of the chromosome and the gene. Amer. Nat. **73**: 300–314.
119. WALDSCHMIDT-LEITZ, E., AND KAFRANYI, E. 1935. Strukturaufklärung des Clupeins. Ztsch. Physiol. Chem. **236**: 181–191.
120. ————, ZIEGLER, F., SCHÄFFNER, A., AND WEIL, L. 1931. Protaminase und die Produkte ihrer Einwirkung auf Clupein und Salmin. Ztsch. Physiol. Chem. **197**: 219–236.
121. WARBURG, OTTO. 1908. Beobachtungen über die Oxydationsprozesse im Seeigelei. Ztsch. Physiol. Chem. **57**: 1–16.
122. ————. 1910. Über die Oxydationen in lebenden Zellen nach Versuchen am Seeigelei. Ztsch. Physiol. Chem. **66**: 305–340.
123. WHITAKER, THOMAS W. 1939. The use of the Feulgen technic with certain plant materials. Stain Tech. **14**: 13–16.

124. Wrinch, D. M. 1934. Chromosome behavior in terms of protein pattern. Nature **134**: 978–979.
125. ————. 1936. On the molecular structure of chromosomes. Protoplasm **25**: 550–569.
126. ————. 1939. The fabric structure of proteins with special reference to the problems of cytogenetics. Proc. 7th Int. Genet. Congr. pp. 329–330.
127. Wyckoff, R. W. G., and Ter Louw, A. L. 1932. A comparison between the ultraviolet microscopy and the Feulgen staining of certain cells. Jour. Morph. **53**: 189–200.
128. Yasuzumi, G. 1933. Über den isoelektrischen Punkt der tierischen Gewebe; I. Bestimmungsmethode des isoelektrischen Punktes von Protoplasten und Verschiebung dieses Punktes der Erythrozyten von Kaninchen durch Formaldehyd. Folia Anat. Japon. **11**: 415–434.
129. ————. 1934. II. Der isoelektrische Punkt der Erythrozyten verschiedener Tierarten. Folia Anat. Japon. **12**: 1–10.
130. ————. 1935. III. Über den isoelektrischen Punkt des quergestreiften Muskels. Folia Anat. Japon. **13**: 55–61.

DISEASE RESISTANCE IN THE VEGETABLE CROPS

J. C. WALKER
University of Wisconsin

INTRODUCTION

The common vegetable crops are, as a rule, of ancient origin; a few have evolved as food plants within the last three or four centuries. There has undoubtedly occurred unconscious or empirical selection for resistance to one or another disease during the long periods in which cultivators have propagated them. There is little or no record of observation during these centuries because of the persistent belief in the fixity of living forms and because of the lack of understanding of the true nature of parasitism and its relation to the cause of disease in living organisms. The last half of the nineteenth century saw rapid changes in attitudes and understanding. In the first decade of this period (1850–1860) De Bary (49) confirmed the previous observations of Prévost (151) and the Tulasnes (210) and settled for all time the fact that certain fungous microorganisms are causal factors in disease production rather than superficial by-products of disease processes resulting from non-parasitic disturbances. Kühn (97) in the same period, through publication of his text-book (1858), stimulated application of remedial measures (some of which had been practiced empirically for centuries), based for the first time on adequate understanding of the underlying principles.

In the field of phytopathology the five decades following De Bary's first work were occupied largely by descriptive science. Scores of new parasitic diseases were described. At the turn of the twentieth century controversy still prevailed over the question of whether bacteria could be the causal factors of disease in plants, and although a virus disease of plants had been clearly recognized by Mayer (119) in 1886, the distinction of the causal entity from the bacteria and the fungi was just becoming clarified.

Relatively little attention was given to disease resistance up to 1900. Most records in this field are fragmentary and are usually found in the annals of plant breeders. Following the devastating epidemics of potato late-blight (*Phytopthora infestans* (Mont.) De

Bary) in Europe and America beginning about 1845, considerable interest in potato breeding was aroused. In 1877 Charles Darwin became interested in the possibility of breeding for late-blight resistance and gave encouragement and some financial aid to James Torbett of Belfast, Ireland, who developed a number of new varieties of that period. In 1880 a committee of the British House of Commons reported that all witnesses concurred in the necessity for the production of new varieties with increased disease resistance, but Parliament turned down requests for financial appropriations. In 1892 Cobb (39), an American who had gone to Australia to join the staff of the Department of Agriculture of New South Wales, was the first to record in analytical detail the differential reactions of wheat varieties to *Puccinia graminis* Pers. At about the same time Ericksson (60) established that several species of rusts were made up of physiologic races which were specialized in their parasitic relations to certain host species or groups of species.

With the rediscovery of Mendel's work in 1901 the beginning of modern genetics furnished a scientific basis for the inheritance of plant characters and for crop improvement through plant breeding. It is a significant coincidence that at this very time Biffen (16) at Cambridge University began his wheat-breeding research in connection with which he observed that resistance to yellow rust (*Puccinia glumarum* (Schm.) Eriks. & Henn.), found in the Rivet variety of wheat was recessive to susceptibility found in Michigan Bronze and Red King, and that in the F_2 generation the population segregated in the ratio of 3 susceptible to 1 resistant while the F_3 families were grouped into approximately one fourth true-breeding resistant lines, one fourth true-breeding susceptible lines and one half segregating lines. When Biffen pointed out that resistance to rust appeared to behave as a simple Mendelian character, it was doubted in some quarters. It is true that Nilsson-Ehle's (132) results at Svalöf led him to interpret inheritance of rust resistance on the basis of the multiple-factor hypothesis, and that only recently Straib (203) has found a type of resistance which is dominant to all other grades of resistance or susceptibility. It should be remembered, however, that at this time our present conception of physiologic specialization in the rusts had not been realized. Ericksson (60) and later Ward (244) had shown several species of rust to consist of races specialized to various host species. It

was not until the work of Stakman and Piemeisel (197) and of Melchers and Parker (120) with *Puccinia graminis tritici* that the study of agronomic varieties in relation to resistance and susceptibility to rust showed that this subspecies of Ericksson was made up of a number of physiologic races defined upon the differential reaction of host varieties rather than of host species. The existence of a large number of physiologic races of *P. glumarum* has since been established in Europe and in America (15, 204).

It is interesting to note the skepticism with which the pronouncement of Biffen was received by plant pathologists. Butler (36) in India pointed out promptly that resistance in wheat for a given rust applies only to a particular variety in a particular locality and does not necessarily hold when the variety is transferred to another locality. Evans (62) in South Africa carried on studies with resistance to *P. graminis.* He used as one parent Bobs Rust Proof, a variety from Australia which remained consistently free from black stem rust at Pretoria although it rusted badly in some other parts of the Transvaal. A local variety, Wol Koren, was used as the susceptible parent. Susceptibility was dominant in the F_1, and, in fact, the hybrid plants were more severely affected than those of the susceptible parent line. Furthermore, the rust from the hybrid plants was infectious to the resistant parent line while rust from plants of the susceptible parent line was not. This indicated that the hybrid plants acted as a bridging host between susceptible and resistant varieties in accordance with the bridging-host theory of Ward which prevailed widely at that time. As a result of these data Evans was inclined to disparage the practical value of breeding for resistance, since it was thought that resistance would decline in new varieties as rapidly as they could be synthesized. Biffen (17) was not discouraged by such disheartening data, for in his experience with varieties and lines of wheat resistant to yellow rust in England the resistant character remained stable for numerous generations, and he pointed to the fact that Rivet, one of the oldest in cultivation, was still highly resistant to yellow rust. There is no question, however, that Biffen's work stimulated greatly the attention of pathologists to the importance of disease resistance in the control of many diseases which did not yield to other established remedial measures. The remarks from now on will be confined largely to the subject as it pertains to diseases of

vegetables, although it should not be forgotten that equally important advances have been made with other food, forage, fiber and ornamental crops, particularly with the cereals, cotton and tobacco.

During the first decade of this century greatest progress was made in the group of diseases known as the Fusarium wilts. Erwin F. Smith (188) had pointed out in 1899 that several hitherto unstudied diseases of such crops as cowpea, cotton, watermelon and cabbage were the result of infection of the root system by soil-inhabiting Fusaria which multiplied in the vascular system—primarily in the xylem—of the plant, causing stunting, chlorosis, vascular discoloration and wilt. The first of these organisms had been distinguished on greenhouse-grown tomatoes in the Channel Islands and named in 1886 by Saccardo as *Fusarium lycopersici* (*F. bulbigenum* C. & M. var. *lycopersici* (Br.) Wr. & Reg. (*F. oxysporum* Schlecht. f. *lycopersici* (Sacc.) S. & H.)). A little later George F. Atkinson (7), then botanist at the Alabama Experiment Station, distinguished the cotton wilt organism and named it *Fusarium vasinfectum* in 1892. In 1899 W. A. Orton joined the United States Department of Agriculture and undertook the study of the wilts of cotton, cowpea and watermelon in the southeastern states. We shall not include here his epochal advances in the development of wilt-resistant cottons upon which the crop in the southern United States and its adaptation to cotton growing in other parts of the world has been built. In the course of his work he noted that watermelon varieties were generally very susceptible to Fusarium wilt (*Fusarium niveum* E.F.S. (*F. bulbigenum* var. *niveum* (E.F.S.) Wr.) (*F. oxysporum* Schlecht. f. *niveum* (E.F.S.) S. & H.)). The African citron, however, was highly resistant. Orton crossed the two forms, and from subsequent segregating generations he selected a watermelon which retained the desirable edible qualities of the latter and the high resistance of citron. This new variety was introduced under the name Conqueror in about 1907 (137). Other important developments started in this decade in the field of disease resistance in vegetables were the beginning of selections of wilt-resistant tomatoes in Tennessee by Essary (61) and of yellow-resistant cabbage (*Fusarium conglutinans*. Wr. (*F. oxysporum* Schlecht. f. *conglutinans* (Wr.) S. & H.)) in Wisconsin by Jones and Gilman (92); the

development of turnip varieties resistant to clubroot (*Plasmodiophora brassicae* Wor.) by Christensen (38), in Denmark; recognition of resistance in bean varieties to two distinct physiologic races of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. by Barrus (10); the study of resistance in potato to late blight (*Phytophthora infestans*) by Jones (91); the development of asparagus resistant to rust (*Puccinia asparagi* DC.) by Norton (133).

THE NATURE OF DISEASE RESISTANCE

Before specific cases of disease resistance in vegetables are discussed, it will be well to outline briefly our understanding of the nature of resistance in plants to various pathogens. It should be borne in mind that disease is the result of the reaction of a variable biologic entity to its environment alone (non-parasitic disease) or to its environment plus a parasitic agent or agents (parasitic disease). In parasitic diseases there are two or more biological entities involved, each responsive to change in its environment. With this concept it is obvious that this phenomenon we call resistance is after all a great array of different reactions between the so-called hosts and their parasites together with the environment. Each case of disease resistance then may be expected to be somewhat different from others. Our knowledge of the fundamental nature of host resistance is still meager, and new lights and new interpretations as they arise from studies of individual cases are continually being added to the slowly evolving picture of one of the most fundamental and interesting phases of biological science.

For the purposes of this discussion it will be sufficient to point out a few representative cases of resistance which illustrate some of the types of host-parasite relationship as we now understand them. We shall look at it from the standpoint of host reaction to the pathogen and leave for later discussion the influence of environment on the picture and the importance of variation in the pathogen itself.

Disease Escape

Disease escape was early recognized by Cobb (39) and by Orton (138). The appearance of resistance to a disease on the part of one variety as compared with another is sometimes due to the fact that for some reason the variety concerned ordinarily escapes the pathogen because of certain environal influences. This

is usually a matter of timing of the growth period of the host with the building up of inoculum and the prevalence of environment favorable to infection. When the same series of varieties is grown in another environment a different varietal reaction picture may prevail. A concrete example of this may be cited. If a series of early-, medium- and late-maturing yellows-susceptible varieties of cabbage are set out on yellows-infested soil in mid-winter in Alabama or Mississippi, the early varieties may show little or no yellows. As the season advances, however, and the conditions for disease development become more favorable with increase in average soil and air temperature, the amount of damage from disease increases in proportion to the time normally required for maturity of the variety concerned. This differentiation is due entirely to the fact that the early varieties have matured before the environment favored infection and disease development, rather than to any quality possessed by such varieties which resist infection or disease development. The same series of varieties set out in July in Wisconsin when the favorable environment for disease is already present, would show quite a different picture, since the disease would develop rather uniformly throughout all.

Cases of disease escape are common and although they are not evidence of true resistance they are often interpreted as such. Thus many of the cases in which varietal resistance is reported may be and often are found to be cases of escape. This accounts in part for the common experience of a given variety becoming recorded as resistant in one locality or in one instance and susceptible in another.

Exclusion of the Pathogen

There are numerous cases in which the fundamental basis of resistance in the variety concerned is one of characters of the plant which preclude or greatly reduce the chances of infection. This type of resistance is in contrast to the other important type in which resistance is manifested through the interaction of host and parasite after infection has taken place. When the barrier of exclusion is overcome by artificial or natural means and infection accomplished, the variety concerned shows the same type of susceptibility as varieties not endowed with this property. The nature of this type of exclusion is not always well understood, and the line of distinction between it and escape is not definite. There are several well defined sub-types which may well be listed, however.

Exclusion by repellance of the insect vector.—When infection is dependent upon an insect carrier of the inoculum from a diseased to a healthy plant, as is commonly the case with virus diseases, there sometimes occur varieties which are resistant to the vector. In the situation of natural exposure they remain relatively free from the disease while other varieties succumb. However, when inoculum is introduced into the resistant variety by artificial means of one sort or another the variety is proved to be fully susceptible. An excellent example of this type of resistance is found in the resistance of the Lloyd George variety of raspberry to mosaic, as shown in the work of Huber and Schwartze (80). Recently, Walker and Larson (232) have observed a case of resistance in the Russet Burbank variety of potato to yellow dwarf which may be of that same type.

Mechanical exclusion.—Cases of mechanical exclusion of the pathogen by characters of the host plant are known. One instance of interest is that of certain lines of maize in which resistance to smut (*Ustilago zeae* (Beckm.) Ung.) consists in characters which provide protection of particularly susceptible regions, such as the lower portion of the internode, from the parasite. The resistance of the tomato fruit to *Macrosporium tomato* Cke. has been shown to increase with age and a correlated increase in resistance of the cuticle to puncture (168).

Chemical exclusion.—The best example of this sub-type is that of the resistance of onion varieties with colored bulbs to certain bulb diseases—onion smudge (*Collectotrichum circinans* (Berk.) Vogl.) and the three neck rots (*Botrytis allii* Munn, *B. byssoidea* Walker, and *B. squamosa* Walker). Associated with the pigments in the dead outer scales are certain water-soluble phenolic compounds—protocatechuic acid and catechol—which diffuse readily into droplets of soil or meteoric water on the surface of the bulb where their toxic effects serve to cause rupturing or stunting or otherwise incapacitate the fungus inoculum, by chemical means. It has been shown that if the dry outer scale barrier is removed so that direct invasion of the living tissue may take place, infection and disease development proceed quite as effectively in colored as in non-colored (susceptible) varieties. This becomes then a case where resistance is purely a matter of chemical exclusion. This case is discussed in more detail later in this paper.

Physiological exclusion.—Exclusion may be brought about by the physiological activity of the host. Numerous observations have indicated a stomatal relation where the pathogen gains entrance through this natural aperture.

Resistance Due to Host-Parasite Interaction

By far the greatest number and the most important cases of disease resistance in vegetables, as in other plants, are the result of a defense by the host after invasion has occurred. Penetration of plants by fungi and bacteria, not able to establish themselves as parasites, is probably much more common than is generally realized. Many years ago Miss Gibson (68), working in Ward's laboratory with the chrysanthemum rust (*Puccinia chrysanthemi* Roze.), found that hyphae of this fungus entered the stomata of many plants which were not hosts. Bond (25) has recently described a similar situation with the tomato leaf-mold fungus (*Cladosporium fulvum* Cke.). Johnson (87) showed that the direct-penetration hyphae of *C. circanans,* the onion-smudge organism, invaded the epidermal cells of several species in which it is unable to establish itself. Johnson (88) has described the effect of subjecting several species to root pressure sufficient to cause excess water in the intercellular spaces of leaves and stems. Under such circumstances necrotic lesions are formed by numerous bacteria which enter the stomata but which are non-pathogenic under ordinary circumstances. The defense mechanism of the resistant host to the invader varies. Some examples of outstanding types may be mentioned.

Resistance due primarily to the cell membranes is illustrated by the resistance of potato tubers to *Pythium debaryanum* Hesse, as reported by Hawkins and Harvey (75). The resistance of cell membranes of the resistant variety McCormick to puncture by the invading hyphae was shown to be measurably greater than that of susceptible forms. Bonde *et al.* (26) state that potato-tuber resistance to the late-blight fungus (*Phytophthora infestans*) is due, in part, to the thickness of the periderm. Resistance due to the interaction of the protoplasts of invader and host is illustrated in the resistance to black wart of potato (37) and in bean anthracnose (101). Between these two extremes there are many cases in which the pathogen invades the resistant host but does not proceed

beyond the early stages; however, neither cell membrane nor cell protoplast reactions have been discerned as accounting for the resistant phenomenon. Cases of this type are best illustrated in the Fusarium wilts, particularly pea wilt (225) and cabbage yellows (5, 191), and in tomato leaf mold (25).

Variability of the Pathogen

The development of disease resistant varieties of plants is concerned first with variation in the host plant and the synthesis of a genetic constitution which includes the combination of resistance with as many other desirable characteristics as possible. The variation of the causal organism in its pathogenic characteristics is equally important and a decisive one insofar as ultimate success is concerned. While the basis of variation in fungi, bacteria and viruses is still in the early stages of exploration, it is clear that some of it is due to segregation following nuclear fusion, some to mutation or saltation, and some to nuclear reassortment. The reader is referred to the discussion of the known facts in this field recently brought together by Stakman *et al.* (196).

It is essential that the type and extent of variability of the causal organism be known as well as possible. In the specific cases to be discussed later it will be seen that the great majority of pathogens concerned in resistance in the vegetable crops are members of the Fungi Imperfecti. In this group variation is more likely the result of mutation or nuclear reassortment. It is important for the breeder to learn whether the variation concerned is one of great change in selective pathogenicity or merely change in degree of virulence. In the vascular *Fusarium* diseases to be discussed, *i.e.*, celery yellows, cabbage yellows, pea wilt, pea near-wilt, cucurbit and tomato wilts, it will be noted that the fungi concerned are quite variable, and in monoconidial lines segregation in virulence commonly occurs. However, in this group there is little tendency for variants to show wide differences in host selectivity. This very fact has been a basic one in the general success which has been attained in the development and stability of varieties resistant to these diseases.

By contrast, the experience with *Erysiphe cichoracearum*, an ascomycete on cucurbits, and *Bremia lactucae*, an oomycete on lettuce, both of which have now or have had at some time a perfect

stage, the distinction between strains is entirely one of host selectivity rather than one of virulence.

The appearance of a new strain, as that of muskmelon powdery mildew and tomato leaf mold, both of which are discussed later, is often attributed to a recent mutation or segregation. On the other hand, it may be entirely a matter of strain assortment. In other words, the new race of leaf mold reported by Alexander (3) may have been present in such minute quantities as to be imperceptible until the variety completely resistant to the old race prevented its perpetuation and permitted only the "new" race to survive and multiply. Circumstantial evidence of this is the fact that the second race came into prominence in Wisconsin greenhouses simultaneously with the same occurrence in Ohio greenhouses. If the second race were a recent mutation it is not likely that exactly the same mutation would occur at exactly the same time in a distinct geographical area. The same explanation might be offered of the new race of *E. cichoracearum* which appeared in the Imperial Valley of California as soon as canteloupe No. 45 was grown almost exclusively (85) although the opinion of investigators in that area is that race 2 is a mutation or was introduced recently.

Of equal importance is the type of variation described by Reddick and Mills (158) and discussed later in the section on potato late blight. In this case the virulence of the pathogen is built up during the growing season as the organism successively attacks varieties of greater resistance. Whether this is a matter of mutation, nuclear reassortment or Dauermodifikation is not clear. It further emphasizes the importance of giving due consideration to variability of the pathogen in any program of breeding for disease resistance.

RELATION OF ENVIRONMENT TO DISEASE RESISTANCE

The importance of soil and atmospheric environment in their influence upon disease development is too well recognized to need amplification here. Likewise, it follows that the variations concerned may be expected to affect the expression of resistance. One of the most important phases of the development of disease resistant varieties is the analysis of the environal relations concerned.

As complete an understanding as possible of the relation of temperature, moisture, light, host nutrition, soil reaction and other factors upon disease development in a susceptible form is an almost

essential prelude to sound procedure in the rating of breeding progenies. If conditions of testing are not accurately controlled, the readings of different seasons are not as readily compared. Average field conditions are not always optimum conditions for disease development. More severe conditions often permit more extensive elimination of susceptible individuals.

Various grades of resistance in a given population often prevail. This is particularly true when the resistant and susceptible individuals do not fall into two well-defined discontinuous classes, as is often the case where resistance and susceptibility are allelomorphs in a single-factor pair, *e.g.*, pea wilt (214) and cabbage yellows (223). More often, resistance is a complex genetic character, and plants of various combinations of genes for resistance may be expected. As a rule, an increasing percentage of the less resistant ones succumb as the optimum environment for disease development is approached. An experimental study of the relation of the more important environal influences will serve as the most reliable guide in the selection of plants for continuance. Those which survive at the optimum may be expected to have the greatest amount of germ plasm controlling resistance. If average field conditions do not provide this environment the artificial approach to it may speed up the breeding program tremendously. In many instances, however, the most highly resistant individuals found may succumb at the optimum; in such cases sub-optimum levels may need to be selected for the testing range.

Examples to illustrate some of the points just made may be found again in the *Fusarium* wilts. In pea wilt (*Fusarium orthoceras* A. & W. var. *pisi* Linford (*F. oxysporum* Schlecht. f. *pisi* (Lindf.) race *1* S. & H.)) resistance and susceptibility fall into two discontinuous classes. Soil temperature is the most important influential factor. At the optimum temperature the resistant individuals withstand the disease quite uniformly while susceptibles succumb rapidly. In near-wilt of pea (*F. oxsporum* Schlecht. f. 8 Snyder (*F. oxysporum* Schlecht. f. *pisi* (Sny.) race *2* S. & H.)) resistance is complex genetically, and various gradations are found. As the soil temperature rises, increasing numbers of resistant individuals succumb until at the optimum all resistants so far studied eventually become diseased though much less rapidly than the susceptibles. Since the optimum soil temperature is much higher than the usual

pea field temperature, a sub-optimum level for selection of resistant individuals is more satisfactory (212).

RESISTANCE IN SPECIFIC VEGETABLE CROPS

Asparagus

Rust (*Puccinia asparagi* DC.). This disease, first reported in the United States in 1896, became of alarming importance in the northeastern States and in California by 1902. Several investigators, including F. A. Sirrine and R. E. Smith (133), pointed out that some varieties, notably the one introduced from Europe as Argenteuil and known also as Palmetto, were more resistant than many in common use and that individuals within a variety often showed striking resistance in comparison with others in the same lot. An intensive breeding program was initiated in 1906 under the auspices of the United States Department of Agriculture in collaboration with the Massachusetts Asparagus Growers' Association. J. B. Norton (133, 134) was in charge and his researches led to a rapid development of the resistant varieties Martha Washington and Mary Washington which since about 1919 have practically replaced all other varieties in the United States. This is one of the notable early successful efforts in the control of a vegetable disease through the development of disease-resistant varieties and in fact the first one in which final results were accomplished rapidly and the products adopted widely.

Norton assembled a large number of stocks and varieties of asparagus from localities in America and from Europe. Within these he selected resistant male and female plants which were tested further as to their ability to transmit rust resistance to their offspring by exposure of hybrid progenies derived from them to rust epidemics. One male plant was finally selected and named Washington; its parentage was unknown. Two female plants, each selected from the Reading Giant variety secured from England, were named Martha and Mary. The F_1 hybrid progeny from Martha × Washington was named Martha Washington, and the seed secured from this pure stand was distributed as Martha Washington Stock; similarly, Mary Washington Stock was distributed from the F_1 stand of Mary × Washington.

It may not be amiss to point out that the work of Norton and its long-standing success rests in part upon the fact, so often lacking

in disease-resistance breeding programs, that the horticultural qualities and the breeding behavior of the plants selected in these respects were given weight equal to that of resistance. As a matter of fact, Norton at the same time contributed to the breeding of asparagus perhaps the chief scientific genetic research on this plant which had been grown as food for many centuries.

Norton was the first to point out that the monocaryotic stage of the long-cycle rust has quite distinct host-parasitic potentialities from the dicaryotic stage. In this autoecious rust he found that plants which were resistant to the infection produced by the dicaryotic mycelium from aeciospores or urediniospores were not resistant to the uninucleate mycelium arising from sporidia produced on teliospores. He was unable to correlate resistance with any morphological character of the host but left the matter open to further study. He did notice that shoots which came up first and were approaching maturity before the rust epidemic developed were affected less seriously than younger ones.

Garden Bean

Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav.). Barrus (10) at Cornell University reported in 1911 observations on variation in susceptibility among bean varieties and announced two physiologic races based on the differential reaction of a series of varieties. In 1915 (11) he reported a strain of red kidney bean which was resistant to both strains of the pathogen, and was designated as Well's Red Kidney. Burkholder (32), his colleague, in 1918 announced the development of a resistant strain of White Marrow by hybridization of the latter with Well's Red Kidney. In the same year Barrus (12) reported detailed results of analysis of the reaction of a large number of varieties to an extensive collection of isolates of the fungus. These isolates were found to fall into two groups which were designated as strains *alpha* and *beta*, respectively. Another strain, *gamma*, was announced by Burkholder (33) in 1923. Leach (101) in the same year described eight physiologic strains distinct from those described at Cornell. While the control of the disease in America has been worked out quite successfully with the growing of fungous-free seed in our western States, it still remains important in Europe where most of the recent research has been conducted. The work of

Böning (27), Budde (30), Doornkat-Koolman (54), Muller (125) and Peuser (141) in Germany and Holland have added greatly to the number of physiologic races.

Burkholder (32, 33) and McRostie (121) showed that resistance to each of the three strains, *alpha, beta* and *gamma,* is controlled by a single gene which is dominant to that for susceptibility. Schreiber (176) in Germany, working with 53 isolations of the pathogen, differentiated 34 physiologic races with the use of 30 host varieties. These were arranged into three groups for each of which a single dominant factor for resistance was found, possibly similar to the three genes described at Cornell. However, in segregating progenies from the crosses Pea Bean 22 × Best of All Wax in which 22 was a Cornell product carrying resistance to *alpha, beta* and *gamma,* ratios were secured which indicated that at least five more dominant genes would have to be assumed to explain them (177). By rigid selection of resistant individuals for six generations, it should then be possible to secure lines homozygous for resistance to all known races.

Common bacterial blight (*Bacterium* (*Phytomonas*) *phaseoli* E. F. S.) *and halo blight* (*B.* (*P.*) *medicaginis* var. *phaseolicola* Burk.). While no varieties have been found which are completely resistant to one or the other of these diseases, an extensive range in the degree of resistance is to be found. Studies of common blight have been made by Burkholder (34), Rands and Brotherton (154) and by Zaumeyer (253). Relative resistance of varieties to halo blight is reported by Burkholder and Zaleski (35), Stapp and Hahne (198), by Kotte (96) and by Ogilvie and Hickman (135). In the United States the following varieties are fairly resistant to common and halo blights: Stringless Green Refugee, Wisconsin Refugee, Idaho Refugee, Robust, Round Pod Kidney Wax, Pencil Pod Wax, Scotia, and French Horticultural; others such as Full Measure are rated as resistant to common blight and very susceptible to halo blight; with still others such as Black Valentine, Tennessee Green Pod and Davis Wax, the reverse is true. In England, varieties Prince, Peerless, Abundant, Black Prince, Black Wonder, Ne Plus Ultra, Superlative, and Unrivalled are comparatively resistant to halo blight. In Germany the halo-blight-resistant varieties listed are Saxa, Konserva, Kaiser Wilhelm, Doppelte holländische Prinzess, Zucker Perl, Mombacher Juni, Marktsieger,

Wachs Black Rom, Wachs Neger, Wachs Flageolet, Schlachtschwert, Krummschnabel, Konservanda, Mohrenweisers. There still remains a great need for improvement in resistance to bacterial blights in many types of bean used in the United States. This is particularly true in the red kidney beans and certain of the types otherwise adapted to long distant shipment such as Bountiful and Black Valentine.

Rust (*Uromyces phaseoli typica* Arthur (*U. appendiculatus* (Pers.) Fr.)). This is an autoecious long-cycle rust. All studies and observations are concerned with resistance and susceptibility to the dicaryotic phase of the pathogen. Fromme and Wingard (66, 67) in Virginia studied the resistance and susceptibility of varieties, listing many in each class. Wingard (252) showed that resistance was a dominant character controlled by a single gene insofar as the pathogen with which he worked was concerned. Wei (246), using the strain secured from Wingard, found that varieties differ in their type of reaction from extreme susceptibility to a high type of resistance in which either hypersensitive necrotic spots or visible sign of infection appeared, while some varieties exhibit reactions of both extremes on the same leaf. He set up six classes of reaction not unlike those in general use in cereal rusts, and classified a large number of varieties on this basis. The pathogen, however, consists of an undetermined number of physiologic races, as demonstrated by the reports of Harter *et al.* (72), Dundas and Scott (57), Parris (140) and Townsend (209). It is to be expected, therefore, that different genes are concerned in the heredity of resistance to different races.[1] The combination of all such characters in one variety is still to be accomplished, and the practical value of so-called resistant varieties will depend upon the races to which they are resistant and the population of races in the locality where they are to be grown.

Powdery mildew. (*Erysiphe polygoni* DC.). The pathogen has a wide host range, including species in numerous families, but it contains many physiologic races. It is probable that more than one race affects beans, and as in bean rust these will be distinguished by the differential reaction of bean varieties. Up to the present no research in this direction has been reported. Differences in resistance of bean varieties have been found in southeastern

[1] Note recent reports by Dundas in Phytopath. 30: 786. 1940.

United States by Cook (42) and by Moore (124), and in California by Dundas (55). The latter has demonstrated that resistance to the fungus with which he worked is controlled by a single dominant gene, not only in common bean but also in tepary bean (*P. acutifolius*), runner bean (*P. coccineus*), yard long bean (*Vigna sesquipedalis*) and cowpea (*V. sinensis*) (56).[1]

Virus diseases. While a number of mosaic viruses are known to be infectious on bean, the two important diseases are common mosaic (*bean virus 1* Pierce) and yellow mosaic (*bean virus 2* Pierce). The first differentiation of varieties on the basis of resistance to common bean mosaic was by Reddick and Stewart (160, 161). Further work in this direction is reported by Pierce (142) and by Smith and Hewitt (189). One important fact which is shown by these studies is that a few varieties or strains are resistant to the point that they do not even harbor the virus as masking carriers. These forms are Michigan Robust, Corbett Refugee, and certain strains of Great Northern selected for this character at the Idaho Experiment Station. Many other varieties, though susceptible, are quite tolerant, and the disease is not ordinarily a serious hazard to their production. Still others, such as the Refugee type used for canning and the dry pea bean type, are extremely susceptible. It is with these more susceptible forms that improvement for resistance has been especially important.

Using Corbett Refugee as a parent in crosses with Stringless Green Refugee, Pierce and Walker (145, 230) developed and introduced two new varieties, Wisconsin Refugee and Idaho Refugee, homozygous to this highly resistant character. Wade and Zaumeyer later introduced U. S. 5 (216) which is also a Refugee type and has the same type of resistance. A number of improved lines of Great Northern have been added by Pierce (143) to those mentioned above. Great Northern is a bean taken over bodily from Indian agriculture in the Dakotas. It is interesting to note that these highly resistant forms were secured by direct selection within the variety. No complete resistance to yellow mosaic has been observed although some varieties are much more tolerant than others, and, generally speaking, it is much more severe on varieties susceptible to common mosaic and vice versa (142).

Pierce (144) studied the inheritance of resistance to common mosaic when Corbett Refugee (CR), Great Northern U.I.1 (GN)

and Robust (R) were used as resistant parents and Stringless Green Refugee (SGR) as the susceptible. Resistance in CR was dominant in all reciprocal F_1 progenies. In GN × SGR the F_1 plants were susceptible, indicating that resistance in GN was different from that in CR. Furthermore, in CR × GN the F_1's were resistant and the segregation in F_2 was nearly the same as in CR × SGR (approximately 88% resistant). Wisconsin Refugee and Idaho Refugee, which were selections from the cross CR × SGR, behaved similarly to CR. In the F_2 of GN × SGR there were 18% resistant plants and 15% in the reciprocal. When R was used as the resistant parent it behaved more like GN than like CR. In R × SGR all F_1's were susceptible and only 12% of F_2's were resistant; in GN × R all F_1's and all F_2's were resistant. In CR × R all F_1's and 79% of the F_2's were resistant. It is pointed out that while a factorial explanation of these observations can not be offered, it is evident that the resistance in CR is much more important to the plant breeder than GN or R, and, as indicated above, three new varieties have already been derived from it. Parker (139) studied the cross R × SGR and reciprocal much more extensively than Pierce. The reciprocals reacted quite differently. In the F_1 SGR ♀ × R ♂ all were susceptible, in the reciprocal 82% were resistant. The influence of the grandmaternal parent was still very evident in the F_2 from SGR × R in which 99% were susceptible while in the reciprocal 56% were resistant. By the third generation there was evidence of marked convergence of results. Because of the different reaction of reciprocals the results could not be explained on a simple Mendelian basis, and it was assumed that the cytoplasm or some extra nuclear inclusion had an effect on the immediate reaction of the plant to the virus, although the convergence of results from reciprocals in F_2 and F_3 pointed to the conclusion that the ultimate control was nuclear.

The common tobacco-mosaic virus (*tobacco virus 1* Johnson) causes local lesions on the inoculated leaves of some varieties of bean and not on others (142, 152). The alfalfa mosaic virus also causes local lesions on some varieties but not necessarily on the same as those susceptible to the tobacco virus (142). Wade and Zaumeyer (217) have shown that in the latter instance resistance is controlled by duplicate dominant genes.

In various parts of western United States the sugar-beet curly-top

virus is a serious pathogen on bean. A Red Mexican variety has been known for some time as resistant to curly top, but it is very susceptible to common mosaic. On the other hand, the common-mosaic-resistant strains of Great Northern are very susceptible to curly top. By crossing Red Mexican with mosaic-resistant Great Northern and selecting from segregating progenies, strains were eventually secured of the Red Mexican type which were resistant to common mosaic as well as to curly top. These were introduced recently as Red Mexican U.I. 3 and 34 (130). At the same time selections toward the Great Northern type led to the perfection of a strain which was resistant to both viruses and similar to the latter variety. This was introduced as Great Northern U.I. 15 (128).

Celery

Fusarium yellows (*Fusarium apii* N. & C. (*F. oxysporum* Schlecht. f. *apii* (N. & S.) S. & H.)). This is a typical vascular fusariosis. In general, green varieties of celery are highly resistant although at high constant soil temperatures they become susceptible, according to Ryker (169). The self-blanching varieties are usually susceptible. However, Nelson (131) and co-workers who have carried out most of the work on this disease have developed a highly resistant line from a survivor of Golden Self-Blanching. This has been named Michigan Golden, and it has proved to be a very satisfactory introduction as to both type and resistance. An improved green type was also derived from a single plant of Newark Market which was introduced as Curly Leaf Easy-Blanching. Nelson found the fungus to consist of two forms which differ in the type of symptom they produce. In one (*F. apii*), chlorosis is produced conspicuously along the veins while in the other (*F. apii* var. *pallidum*) the veins remain green until the leaf is entirely chlorotic. Resistance in the varieties mentioned is similar to both forms.

Cruciferous Vegetables

Yellows (*Fusarium conglutinans* Wr. (*F. oxysporum* Schlecht. f. *conglutinans* (Wr.) S. & H.)). This disease is so far entirely American. The causal fungus, a typical vascular Fusarium, is restricted to members of the species *Brassica oleracea* L. which contains cabbage, cauliflower, kale, brussels sprouts, broccoli, kohlrabi and the wild cabbage of Europe (241). All these hosts are sus-

ceptible but they contain varying percentages of resistant individuals. The organism is more uniform in its pathogenicity than many vascular parasites and no evidence of physiologic races has been found (19). Resistance of two types occurs. That which is controlled by a single gene dominant over susceptibility was the first single-factor difference to be found in the rather widely occurring cases of resistance to this class of pathogens (221). The same gene was found in wild cabbage (223) and in brussels sprouts and kohlrabi (21). This gene did not account for all cases of resistance, and a type with a more complicated heredity was distinguished by Anderson (4) and by Blank (20) in certain lines of resistant cabbage. The first type is referred to as Type A and the second as Type B (227).

Numerous varieties of cabbage resistant to yellows have been developed by selection within standard varieties. Since cabbage and its close relatives are naturally cross-pollinated the commercial varieties are as a rule heterozygous for many characters. The genes for resistance are well distributed although older commercial varieties used in America usually contain a small percentage of plants carrying them. Since the first varieties were developed without knowledge of the genetics of resistance no check was made on whether Type A or Type B genes were present. Thus the first variety, Wisconsin Hollander (92), was found later to contain only genes for Type B resistance (4). In the second variety, Wisconsin All Seasons (93), about 20% of the resistant plants contain Type A genes, and in the remainder only Type B resistance occurred (20). In varieties developed and introduced after 1926 Type A resistance was incorporated (224, 231, 238). Breeding for resistance in other forms of *B. oleracea* except kale has not been undertaken largely because the disease is not important where the crops are grown or because, as in cauliflower, varieties naturally contain a fair degree of one or another type of resistance. In the Petaluma district of California yellows was severe on kale, and Kendrick has initiated selection of resistant lines in that area (94).

Individuals or varieties of cabbage with Type B resistance succumb more readily to the disease as the soil temperature increases (239), and in the field such varieties as Wisconsin Hollander commonly show large percentages of mildly affected plants. Plants or varieties with Type A resistance are much more stable in this char-

acter and fungus invasion is not great nor are typical symptoms produced even at high constant soil temperatures. This soil temperature differential has become a very useful criterion in sorting out individuals in breeding progenies. By exposing young seedlings in infested soil to a constant soil temperature at 24° C. for two to three weeks it is usually safe to assume that all survivors are homozygous or heterozygous for Type A resistance.

Histological studies have indicated no morphological characteristics of resistant roots which are responsible for resistance. Slight invasion of the cortex of root or hypocotyl of resistant plants occurs but no establishment in the vascular system occurs in Type A resistant plants. In Type B plants this does occur but in a more limited way than in susceptible plants. No correlation between the reaction of extracted sap from resistant and susceptible plants and their reaction to the pathogen has been found (208).

Clubroot (*Plasmodiophora brassicae* Wor.). The clubroot disease has been destructive for centuries on various crucifers. Most of the attention to the question of host resistance has been in connection with the cruciferous root crops, turnip and rutabaga. Differences in varietal reaction of these vegetables have been noted from time to time for a century or more. In recent years there have been developed several resistant forms which have come into extensive use. Of these the May, Bruce and Immuna turnips and the Herning and Studsgaard strains of the Bangholm variety of rutabaga are outstanding (136, 228). In the various vegetables of *Brassica oleracea* no consistent resistant forms have been found or developed with the exception of some highly resistant strains of marrow kale (116).

The existence of physiologic races of the pathogen has been suggested by several workers and this seems the more probable since Walker (228) found quite different reactions of certain varieties of rutabaga in America from those reported from the same stocks in the British Isles. Rochlin (163) attributed the high resistance of black mustard (*Brassica nigra*) to its high content of the volatile oil, allyl isothiocyanate, and believed there was a distinct correlation between the resistance of various species of crucifers and their oil content. Considerable doubt has been thrown on this theory since Walker (226) found that some collections of *B. nigra* are very susceptible. While working with the same materials Walker, Link and Morell (237) found no correlation between oil content and resistance of various lots of black mustard.

Cucurbits

Fusarium wilt of watermelon (*Fusarium niveum* E. F. S. (*F. oxysporum* Schlecht. f. *niveum* (E. F. S.) S. & H.)). As already indicated, Orton developed a wilt-resistant watermelon variety from a cross between a cultivated form and an African citron and introduced it under the name Conqueror. This variety never came into wide use, due in part to the fact that its horticultural type did not suit changing market needs. Porter and Melhus (149) many years later found it only slightly more resistant than a quite susceptible variety, Kleckley Sweet, in southeastern Iowa. These workers selected one new variety from Kleckley Sweet which was introduced in 1931 as Pride of Muscatine. Two other varieties were selected from crosses with Conqueror and introduced the same year as Iowa Belle and Iowa King. In 1936 Walker (242) announced a new variety, Leesburg, selected from Kleckley Sweet under Florida conditions, and two new strains, Improved Kleckley Sweet No. 6 and Improved Stone Mountain No. 5, were released in Iowa. The following year Porter (147) released a resistant strain of Klondike known as Klondike R 7 which he developed in California from a cross of Iowa Belle and Klondike. A recent development from Australia under the name Hawkesbury has found favor in Virginia (43).

The watermelon wilt organism is quite variable in virulence. Several physiologic races have been designated by Sleeth (187) but the differences between them are primarily in virulence. On the other hand, Porter (147) points out that this degree of variability has not interfered seriously with the development of commercially successful varieties. Wilson (251) emphasizes that the host is susceptible at various stages in its development but that effective resistance increases with the age of the plant. The inheritance of resistance was studied by Bennett (13) who concluded that it is governed by a number of genes, some of which are probably cumulative in their effect.

Fusarium wilt of muskmelon (*F. bulbigenum* C. & M. var. *niveum* Wr. f. 2 L. & C. (*F. oxysporum* Schlecht. f. *melonis* (L. & C.) S. & H.)). This disease, described recently by Leach and Currence (102), is destructive to muskmelon in Minnesota and New York. Persian Honeydew, Honeyball and Casaba varieties are relatively resistant but the varieties suited to the conditions where the disease

resistant lines. These were next reduced to two, Nos. 39 and 40, which in recent trials have not only outyielded susceptible varieties, although the latter were protected with eight applications of Bordeaux mixture during the season, but produced a higher percentage of fancy and choice fruits (165).

Lettuce

Downy mildew (*Bremia lactucae* Reg.). Five physiologic races of the causal fungus were described by Jagger and Whitaker (84), four of which were found in California, one in England. Schultz and Röder (181) found two races in Germany. Most commercial varieties of lettuce are susceptible but occasional resistant types were found both in California and in Germany. In all cases the varieties carrying resistance were of European origin and were of the most primitive type. Resistance to each of two of the known physiologic races was shown to be controlled by a single dominant gene. A number of commercially desirable strains resistant to four of the five races of the pathogen in California have been released.

Brown blight. This disease formerly occurred very destructively in southern California and in Arizona. Its causal factors have never been determined but they are definitely soil-borne. Jagger (82) tested over 100 varieties of lettuce on diseased soil and only two, Big Boston and Chavigne, remained disease-free. When several hundred second-generation plants of Chavigne × New York were tested on severely infested soil the segregation was approximately 3 resistant to 1 susceptible. In 1924 about 100 resistant survivors were selected from a badly diseased field of New York, the most widely grown type in southern California. Some of them were off-types and others fairly close to the standard New York. The progenies of many of these were highly resistant and three of the most desirable type were increased. Eventually only one was retained and it was released in 1926 as Imperial No. 2. This strain, or strains derived from it, is used almost exclusively in the Imperial Valley at the present time, and widely throughout the United States.

Onion

Smudge (*Colletotrichum circinans* (Berk.) Vogl.) and *Neck rots* (*Botrytis allii* Munn; *B. byssoidea* Walker; *B. squamosa* Walker). These diseases affect the bulbs of onion at harvest time or slightly

ontinue as storage rots. It has long been known that
*sarium*which yellow or red pigments are developed in the scales
ess subject to these diseases than non-pigmented ones.
fore and c ge organism infects the fleshy scale by penetrating the
rieties in , ectly, the mycelium digesting first the subcuticular cellu-
e much the outer wall of the epidermal cell and then penetrating
ie smud ng tissue (219). As the onion plant approaches maturity
ticle di more outer scales have formed which contain no living cells
se of t time but due to the tensile strength of the desiccated tissue
derlyin as papery, brittle, closely clasping outer dry scales. In the
e or w and red varieties these outer scales contain most of the color-
the matter; in the underlying fleshy scales the color, yellow or red,
na a sap-soluble material in the cells of the outer epidermal layer.
lc he smudge organism as a rule becomes established in these outer
ry scales, especially in the non-pigmented variety. In the yellow and red forms the varietal resistance is evident at this stage by the practical absence of the disease even in the outer dry scales. Walker and associates (220, 234, 235) showed that the fleshy scales of all onions contain volatile materials, some of which are probably the sulfur oils, extremely toxic to the smudge organism and some other fungi. However, this did not explain the varietal resistance of colored varieties. The watery extracts from the dry outer scales showed a correlative difference, however; that from white scales supported good germination and growth of the smudge and neck-rot organisms; that from colored scales prevented germination, caused germ tubes to rupture, or retarded growth. Another onion-bulb pathogen (*Aspergillus niger* van Tiegh.) which attacks white and colored bulbs alike was not materially inhibited in the colored-scale extract. It was thus evident that the water-soluble materials which diffused into the droplets of water on the surface of dry outer scales contained toxic materials which inactivated the invading fungi sensitive to them.

When the outer scales were removed the whole picture was changed. The materials present in the living epidermal cells did not diffuse into the external moisture containing the inoculum. Spore germination of the smudge organism occurred normally, cuticular penetrations proceeded similarly in colored and white bulbs, and digestion of the subcuticular wall followed. The action of enzymes in advance of the fungus was sufficient to break down the

color compounds in advance of the mycelium and there was no opportunity for them or any closely allied compounds to exclude the pathogen.

Thus resistance is effective only as it is expressed in the dry scales of colored varieties. It is, furthermore, very closely associated with the coloring materials. A chemical study of these toxic scale extracts by Link, Angell and Walker (6, 112, 113, 114, 236) led to the isolation and identification of two phenolic colorless compounds associated with the color compounds which are water-soluble and responsible in a large measure for the resistant character of the colored outer scales. These substances are protocatechuic acid and catechol.

In association with these workers, Rieman (162) investigated the genetics of onion scale pigmentation. Using Red Globe, Yellow Globe and White Globe varieties, he identified genes as follows: *I*, a gene for incomplete inhibition of color; *i*, a gene allowing expression of color; *W*, a gene for red pigment; *Wy*, a gene for yellow pigment, *w*, a gene for white. The indications are that, *W*, *Wy* and *w* are multiple allelomorphs independent of the factor pair *I* and *i*. *W* is dominant to *w* and to *Wy*, *Wy* is dominant to *w*; *I* is incompletely dominant to *i*. White bulbs may have the genes *w i* or *II*. When the genes *I i* are present with *W* or *Wy* the incomplete dominance of *I* produces an intermediate scale color and an intermediate degree of scale resistance to smudge. Inasmuch as color is so closely associated with resistance, although the toxic materials isolated are colorless, the question is left open whether white resistant onions can be secured. If the phenolic compounds are formed as precursors of the pigments, then the *I* gene may possibly permit the formation of the toxic materials and suppress the formation of color. If the phenolic substances are decomposition products of the pigments the possibility of securing white resistant bulbs is very remote. The close association of color with resistance and the fact that intermediate gradations of color could be explained on a factorial basis has made it possible to give also a factorial explanation to various intermediate types of resistance.

Pink root (*Phoma terrestris* Hansen). Porter and Jones (148) have shown that Sweet Spanish is the only variety tested which shows any resistance to this disease, and only moderately so. *Allium fistulosum* (Nebuka type), *A. porrum* (Giant Musselberg variety)

and *A. schoenoprasum* are highly resistant. The possibility of introducing resistance from *A. fistulosum* into *A. cepa* through an inter-species cross has been pointed out by Jones (89), and work in this direction is in progress.

Smut (*Urocystis cepulae* Frost). No evidence of resistance within *Allium cepa* to this important disease has been found. Felix (65) pointed out, however, that *A. fistulosum* showed marked resistance. Evans has studied the host-parasite relationship in both *A. cepa* and *A. fistulosum* (63, 64). He showed that although a high percentage of infection of the latter may occur, the cotyledon resistance which develops with age of the organ in *A. cepa* is much more pronounced in *A. fistulosum,* with the result that most of the infection occurs in the upper part of the cotyledon and subsequent invasion of the true leaves is thus precluded. If this type of resistance can be transferred to *A. cepa* a commercially satisfactory resistance to smut should result.

Yellow dwarf. Henderson (77) tested many varieties of onion for resistance to this virus disease. He found only one, Riverside Sweet Spanish, which did not become diseased when others were heavily infected.

Garden Pea

Wilt (*Fusarium orthoceras* A. & W. var. *pisi* L. (*F. oxysporum* Schlecht. f. *pisi* (Lindf.) race *1* S. & H.)). This disease is a typical vascular fusariosis. Linford (109), when he first distinguished it, noted the striking difference in resistance and susceptibility of varieties. Surveys of many varieties have shown that a large percentage of them fall into the highly resistant or highly susceptible class and that usually plants in a mixed or a segregating progeny fall into distinct discontinuous classes, resistant or susceptible (218, 222). Wade demonstrated that resistance is dominant to susceptibility and is controlled by a single gene (214). Snyder (193), in a study of the variability of the causal organism, showed a wide range of virulence between isolates and also a good deal of variability within monoconidial lines. However, this did not affect the status of resistant varieties since even the most virulent forms did not affect them. Although many naturally resistant varieties occur, some of the most desirable ones in use in the United States are completely susceptible. Thus many new resistant forms have been developed in recent years, chiefly by hybridization and subsequent selection (51, 229, 240).

Linford made extensive studies of the relation of the organism to resistant and susceptible host plants. He concluded that the resistant quality was a character of the root system. Walker (225) studied the relation of the pathogen to resistant and susceptible roots. In the latter, invasion of the root tip takes place as in cabbage yellows, with more or less breakdown of the cortical tissue and rather prompt invasion of the xylem. In the resistant root, invasion of the cortex of the young root is meager and was never seen to reach the stele. A study of root extracts of resistant and susceptible Alaska peas and of nutrient solutions in which the plants had grown was made by using them as a medium for culturing the parasite. Usually the weight of the fungus was less in the resistant as compared with the susceptible extract. There was an indication of the presence of specific inhibitive materials in the resistant extract since when another pea-root parasite, *Fusarium martii* A. & W. var. *pisi* Jones (*F. solani* (Mart.) A. & W. var. *martii* (A. & W.) Wr. f. *2* Snyder), was used, no difference was noted.

Near-wilt (*Fusarium oxysporum* Schlecht. f. *8* Snyder (*F. oxysporum* Schlecht. f. *pisi* (Sny.) race *2* S. & H.)). This disease is a second vascular fusariosis of the pea. The fungus invades the root system and travels up the xylem much as the wilt organism does, except that the former progresses much farther up the stem (195, 212, 213). The disease develops much more slowly than wilt. There is no correlation between resistance of the host to wilt and near-wilt. In fact, all wilt-resistant varieties studied are quite susceptible to near-wilt except Horal and Roger's K. Furthermore, the resistant and susceptible plants do not fall into two discontinuous classes as in wilt, and even the most resistant varieties succumb eventually when they are exposed to extremely favorable conditions for the disease. There is promise, however, of improving varieties for resistance to near-wilt by hybridization and selection.

Virus diseases. A number of distinct viruses are infectious to the garden pea. In some, complete resistance is shown by certain varieties; in others, differences in degree of resistance occur between varieties that are affected. Stubbs (207) found all varieties he tested to be susceptible to *pea virus 1* Stubbs, while the following varieties were not infected by any of the three strains of *pea virus 2* Stubbs: Perfection, Giant Wonder, Abundance, Ashford, Nott's Excelsior, Hundredfold. Murphy and Pierce (129) reported that

the following varieties were not infected by *pea virus 3* Pierce: American Wonder, Canners Gem, Dwarf White Sugar, Early Bird, Horal, Hundredfold, Laxton's Superb, Little Marvel, Morse Market, Nott's Excelsior, Onward, Perfection, Premium Gem, Rice's 13, Surprise, Thomas Laxton, White Marrowfat, Wisconsin Early Sweet, Zwaan's Banquet, Mackay, and Tom Thumb. Zaumeyer (215, 254) found that *pea virus 4* Zaumeyer, *pea virus 5* Zaumeyer, *alsike clover virus 1* Zaumeyer, *alsike clover virus 2* Zaumeyer were not infectious to Horal, Little Marvel, Perfection, Surprise and Wisconsin Early Sweet. Thus Perfection is resistant to six distinct viruses, and Little Marvel, Horal, Surprise and Wisconsin Early Sweet to five. Of these, Zaumeyer and Wade (255) found Little Marvel, Surprise, and Horal were among those mildly affected by *pea streak virus 1* Zaumeyer.

Spinach

Mosaic or blight. Although spinach is susceptible to several distinct mosaic viruses the most common and destructive disease in the eastern United States is that due to cucumber mosaic virus (78). At the Virginia Truck Experiment Station two very widely used varieties have been developed which are highly resistant to cucumber mosaic. An importation of a wild Asiatic strain by Frank N. Meyer of the U. S. Department of Agriculture was highly resistant to mosaic. This strain, known as Manchuria, was crossed with Bloomsdale Savoy, Round Thick-Leaf Winter, Flanders, and Long Standing. Selection for resistance and Savoy type led to the development of Virginia Savoy (190). A later cross between Virginia Savoy and King of Denmark led to the development of Old Dominion. This is one of the outstanding early cases of the control of a virus disease of a vegetable through the development of resistant varieties.

Sweet Potato

Stem rot (*Fusarium batatis* Wr. (*F. oxysporum* Schlecht. f. *batatis* (Wr.) S. & H.)). Various varietal comparisons of sweet potato varieties have shown that a wide range of resistance and susceptibility to stem rot occurs (73, 74). Harter and Whitney tested most of the commercial varieties used in the United States by growing them for four consecutive seasons on infested soil in Delaware. No varieties were completely resistant, but Creola, Dahomey, Haiti,

Key West, Pierson, Pumpkin, Red Brazil, Southern Queen, Triumph, White Yam, and Yellow Strassburg were so highly resistant that a normal crop was produced on badly infested soil. On the other hand, such popular commercial varieties as Nancy Hall, Yellow Jersey, Red Jersey, Porto Rico, Gold Skin, Georgia and Big Stem Jersey were very susceptible.

Surface rot (*Fusarium oxysporum* Schlecht.). The disease affects all varieties, but the Jersey types appear to be most susceptible. Lauritzen (99) developed a strain of Yellow Jersey by selection which had high resistance to surface rot. The resistant clone has a darker colored skin and a higher degree of russeting than the susceptible variety, and it withstands shriveling to a greater extent in storage. The resistant strain was named Improved Yellow Jersey.

Soil rot or pox (*Actinomyces* sp.). In general, varieties with red skins are more resistant than those with white or yellow skins (73).

Eggplant

Silayan (186) reports a variety highly resistant to *Phomopsis vexans* under the name Pampanga White. Bacterial wilt (*Bacterium* (*Phytomonas*) *solanacearum* E.F.S.) is a very serious disease in the tropics. Roque and Adsaur (166) in Puerto Rico found a native, non-commercial variety which is highly resistant. Crosses were made with several commercial varieties, and in 1939 two satisfactory highly resistant commercial varieties were announced. One of these, Puerto Rico Beauty, has a dark purple fruit and is suited for the export trade; the other, E. 12, is pink-fruited and is adapted to local market needs.

Pepper

Malabanan (117) states that sweet varieties are subject to anthracnose (*Colletotrichum nigrum* E. & H.) while hot varieties are resistant. Snyder and Rudolph (194) noted that the variety Anaheim was much more severely attacked than Red by *Verticillium albo-atrum* R. & B. Holmes (79) has studied the inheritance of host reaction of pepper varieties to tobacco mosaic virus. A gene, L, which controls local necrotic reaction is dominant over l^i which controls imperfect localization and l which controls systemic mottling and stunting. The commonest genetic constitution of commercial large-fruited, sweet varieties, as California Wonder, World Beater and Ruby King, is the homozygous recessive $l\ l$, and they are very

susceptible to severe damage. Long Red Cayenne and Sunnybrook Cheese are homozygous for incomplete localization ($l^1 l^1$) and are not seriously damaged. Tabasco is homozygous for complete localization. Since the three genes are members of an allelic series it is possible to develop highly resistant lines in the susceptible varieties, and such a breeding program is reported to be under way. Roque and Adsuar (167) report a mosaic disease distinct from that due to *tobacco virus 1* in Puerto Rico, to which all commercial varieties of sweet pepper are susceptible. One native variety of hot pepper which is highly resistant has been found, and this may be used as a resistant parent for the development of resistant sweet strains.

Irish Potato

Wart (*Synchytrium endobioticum* (Schlib.) Perc.). Potato culture in many parts of Europe is harassed by the inroads of this disease of roots and tubers. Described originally in Hungary in 1895, it has now become widely distributed and occurs in many parts of the world. In America it has been found in Newfoundland, while in the United States it is confined by quarantine to limited areas in the Appalachian plateau. Since the organism remains viable for long periods in infested soil, the use of resistant varieties is the only satisfactory control. It was recognized early in the century that many varieties of potato are resistant to the pathogen. By 1918 Great Britain had a well-established testing station at Ormskirk, England, where standard varieties and new seedlings were tested and evaluated as to the degree of their resistance to wart (192). This service has been inaugurated and maintained in many of the European countries. It is now usually supplemented by a laboratory test in which the young sprouting eye tissue of a tuber of the clone in question is exposed to a fresh suspension of zoospores. Since the meristemic tissue is the most susceptible, reaction is prompt, and a reliable index of the behavior of the variety in the field can be obtained rapidly (107). Varieties exhibit various grades of resistance which are sufficiently constant within the clone to permit varieties to be classified on that basis (70, 95). Even in the most resistant varieties penetration of the cuticle and the epidermal cell occurs but the host-parasite relation is such that the invading protoplast dies (37). In some varieties a sufficient number of the surrounding host cells die also to give a visible necrotic lesion. In

some, host reaction results in very slight hyperplasia and some spore formation of the pathogen without any commercial damage other than that occasioned by the fact that such varieties perpetuate and distribute the causal organism.

Many standard varieties in use in the United States are highly resistant to wart. Among these are Irish Cobbler, Spaulding Rose, White Rose, Russet Burbank, and Green Mountain. Very susceptible varieties are Rural New Yorker, Russet Rural, Early Ohio, and Bliss Triumph. In most European countries black-wart resistance is a prime requirement of new varieties which are developed through breeding.

The inheritance of resistance to black wart has been studied by Lunden and Jørstad (115) in Norway, by Salaman and Lesley (171), by Black (18) and by Collins (41) in England. The genes for resistance may be different in different varieties. At least three genes are concerned. In some varieties, such as Hindenburg and Jubel, which are used extensively because of their resistance to common scab, a single dominant gene controls resistance. In other varieties two complimentary dominant genes account for resistance.

Late blight (*Phytophthora infestans* (Mont.) De Bary.). It has already been mentioned that the late-blight epidemics in the middle of the nineteenth century stimulated many potato breeders to search for and develop blight-resistant varieties in Europe and America. Among these was Chauncey Goodrich of Utica, N. Y., who imported a small quantity of potatoes for breeding purposes from South America in 1851. When Jones (91) went to Europe in 1904 he found a great deal of attention being given in the British Isles and on the Continent to the improvement of the potato for late-blight resistance. At that time numerous varieties were being grown which showed relatively more resistance than others either in leaf or in tuber, but none was really highly resistant or immune. There was a general opinion that resistance and vegetative vigor were closely related. The experience for several decades had been that varieties gradually became less vigorous with use, and that their late blight resistance also declined. Thus the first resistant variety produced which secured wide acclaim was Magnum Bonum, derived from a cross between Early Rose, imported from the United States, and Victoria. It was introduced in 1876 and remained in high favor until about 1890. It was replaced in favor by Up-to-Date in En-

gland and by Richter's Imperator in Germany, each of which declined in turn. The relation of viruses to degeneration of potato was not understood and they undoubtedly had much to do with the decline of these varieties. Stuart (206) tested many varieties brought from Europe by Jones and he found that in general the Dutch, German and English-Scotch varieties showed less tuber rot than did American and French varieties. The potato-breeding program initiated by the United States Department of Agriculture in 1910 under the leadership of Stuart has been carried on since that date, one of the objectives being the improvement of potatoes for blight resistance. Likewise, work in the same direction has been carried on in Russia under Bukasov (31) and Sidorov (185), at the Biological Institute at Dahlem, Germany, under Müller (126, 127), at the Kaiser Wilhelm Institute at Muncheberg, Germany, under Schick (172, 173, 174) and Lehman (105, 106), and at Cambridge University under Salaman (170).

It has now become generally recognized that all varieties of *S. tuberosum* are susceptible in some degree to *P. infestans* although in many resistance is high enough to be of commercial value. Until recently not any of the resistant varieties produced or introduced in the United States has been satisfactory in horticultural type and has therefore come into general use. The work of Stevenson (26, 201, 202) and associates has shown that this type of resistance is inherited as a recessive character, and that probably several genes are involved. Since susceptible varieties may carry genes for resistance in the heterozygous condition, resistant seedlings may appear in the hybrids from two susceptible parents. An example of this is the recently introduced variety, Sebago, which was derived from a cross between two susceptible varieties, Katahdin and Chippewa (199). Foliage blight resistance is not necessarily controlled by the same genes as tuber-rot resistance and the two types do not always occur in the same clone (26).

The high resistance of certain wild species of *Solanum* was recognized many years ago but the possibility of securing resistance through species crosses was not followed up aggressively. Within the last ten years there has been renewed effort in this direction in both Europe and America. In the United States this phase of the program is under the leadership of Reddick (155, 159). Major attention is being given to crosses of *S. tuberosum* with *S. demissum.*

Some forms of the latter are practically immune to blight although, as pointed out by Schick and Schafer (174), some may be susceptible. The immunity in *demissum* is dominant to susceptibility. The procedure being followed now quite generally is to back-cross the F_1 to *tuberosum*, eliminate susceptible segregates, and continue the procedure with successive back-crosses. By this procedure there is reason to believe that in the near future good commercial sorts with the immunity of *demissum* will be forthcoming.

Variability in *P. infestans* was first noted by Giddings and Berg (14, 69) who designated strains which affected only potato severely as contrasted with those affecting both potato and tomato. About 1930 Müller (126, 127) noted a difference in the reaction of breeding stocks to *P. infestans* and on further study concluded that physiologic races differing in their pathogenicity on potato existed. These differences, however, were primarily in virulence, the most virulent strain attacking severely strains of the host which were quite resistant to other strains. Strains varying in pathogenicity have been noted by others in Europe and America (14, 105, 106, 157, 173, 174). None of the strains, however, collected in Germany or elsewhere has been infectious to the immune lines of *demissum* or to immune lines derived from *tuberosum* × *demissum*.

The nature of pathogenic variability has been studied critically by Reddick and Mills (122, 123, 158) and their work is an important chapter not only in the annals of late-blight resistance but in the general field of the variability of pathogenic organisms. They noted in field plots that when *P. infestans* developed first in a very susceptible variety and spread naturally to varieties with increasing degrees of resistance, the virulence of the strain increased and under such circumstances the highly resistant forms which ordinarily remained free from infection were attacked. This property of the organism to change in virulence under the influence of the substrate was carefully checked under controlled conditions in the laboratory. Further study of the matter in relation to tomato has shown that the potato strain weakly pathogenic to tomato may by successive transfers be stepped up to a high degree of virulence to tomato. Thus the tomato strains of Giddings and Berg are interpreted as potato strains in which virulence for tomato has built up during the current season. This coincides with the common observation that late-blight epidemics on tomato follow those of potato by several weeks during

which virulence for tomato may have been built up. This is the first instance where such rapid changes of virulence under natural conditions has been so clearly demonstrated. It may explain why such older varieties as Magnum Bonum gradually lost their resistance to late blight. It has an extremely important bearing on the future stability of resistant forms of *tuberosum* such as Sebago and it increases the importance of *demissum* hybrids, in which immunity to all known forms of *P. infestans* still exist, in the permanent control of late blight through disease resistance. In a recent study of the host reaction of *demissum* type, Bordukova (29) has shown that necrosis sets in rapidly upon invasion by the pathogen making conditions immediately unfavorable to the latter, while in susceptible varieties no such initial reaction to the parasite occurs and deep-seated infection of the tissue is established promptly.

Common scab (*Actinomyces scabies* (Thaxt.) Güssow.). Many early workers have carried out comparative trials with potato varieties on scab-infested soil. It has long been recognized that some standard varieties show a commercially important degree of resistance. However, for many soil and climatic conditions satisfactory early and late scab-resistant varieties do not exist. The disease is quite responsive to soil environment and a given variety reacts quite differently from season to season in the same locality (233). Schlumberger (28, 175), who conducts official varietal tests for scab resistance in Germany, also found the variation from year to year a serious handicap in giving specific ratings to varieties.

A few highly resistant varieties have been developed in Europe which are of value principally for breeding purposes. Chief of these are Richter's Jubel and seedlings derived from it (Hindenburg, Erdgold and Arnica), as well as Ostragis, Ackersegen, Dauerragis, Aal, Treff As and a cross between Hindenburg and Centifolia. Russet Burbank and Russet Rural are the most resistant commonly used American varieties. Clark (44, 45) and associates studied the breeding behavior of some of these and their results indicate that Hindenburg and Ostragis are homozygous for resistances while Jubel is heterozygous. Moreover, the susceptible variety Katahdin apparently carries a recessive gene for resistance in heterozygous condition. Leach and associates (104), studying the heredity of scab resistance in 37 hybrid families, found Jubel and selection 5–14–8–1 to have high breeding value. Variation in the selective

pathogenicity of strains of *Actinomyces scabies* has been described in Holland by De Bruyn (50) and in the United States by Leach *et al.* (103). Darling (48), in a study of the nature of scab resistance, found no consistent relation between scab resistance and russet type of skin or morphology of stomata. However, lenticels were larger, rounder and more loosely arranged in susceptible than in resistant lines. Suberization of the periderm took place earlier and extended farther into the lenticels in the resistant tubers. Reddick (156) has recently pointed out the possible value of certain wild species of *Solanum* as breeding parents.

Virus diseases. The breeding of varieties resistant to one or another virus disease is under way in many laboratories in America and in Europe. One obvious retarding influence is the lack of complete understanding of the variation in strains of viruses and the distinction of one virus from another. Mention may be made first of *latent mosaic,* the virus of which is referred to commonly as X-virus. Few varieties are completely resistant but the reaction of different ones varies greatly. Schultz *et al.* (178, 179) classify varieties into three groups on the basis of reaction to X as follows: (1) those highly resistant as S41956; (2) varieties resistant in field tests but contracting latent mosaic in tuber and shoot grafts or manifesting the disease as top necrosis; (3) symptomless or masked carriers. All standard American varieties are of the third class. Varieties of types 1 and 2 would be more desirable because of the fact that those in class 3 are subject to mosaic diseases in which virus X is one component. Stevenson *et al.* (200) have shown S41956 is immune to six strains of virus X and that the inheritance of this immunity is explained on the basis of the usual type for autotetraploids where two dominant complementary genes are necessary for expression of the character. Mild mosaic is another serious disease on American potatoes. Evidence that it may be eventually controlled by resistance is that the United States Department of Agriculture has already released three varieties highly resistant to it, Katahdin, Chippewa and Sebago (46, 199). Veinbanding mosaic may eventually be controlled in the same way since a wide variation in reaction to potato seedlings was found by Schultz *et al.* (179) and by Jones *et al.* (90). The virus of this disease, referred to by some as Y, when present in masked carriers of X, combines with the latter to cause rugose mosaic.

Tomato

Fusarium wilt (*Fusarium lycopersici* Sacc. (*F. bulbigenum* C. & M. var. *lycopersici* (Brushi) Wr. & Rg.) (*F. oxysporum* Schlecht. f. *lycopersici* (Sacc.) S. & H.)). As already pointed out, Essary (61) started the selection of wilt-resistant tomatoes in Tennessee before 1910. A program was initiated a little later in Louisiana by Edgerton and Moreland (58). The early success at these two centers stimulated work in many states where selection for resistant varieties adapted to local requirements was carried on. The most extensive program was that in the United States Department of Agriculture led for many years by Pritchard (153) who introduced several widely used resistant varieties. Other varieties have been developed at Kansas (250), Illinois (81), Michigan (205) and California (184).

None of these is completely resistant. Wellman (248) has recently improved the method of studying host resistance. Recent work by Wellman and Blaisdell (249) confirms that of Haymaker (76) and shows, further, that a considerable range of pathogenicity is found when isolates of the pathogen are compared, and that saltation within isolates occurs. No wide divergence in selective pathogenicity was noted, however. The recent discovery by Bohn and Tucker (24) of a strain of red currant tomato (*Lycopersicon pimpinellifolium*) which was completely resistant to wilt has opened up a new range of possibilities in the development of highly resistant commercial forms since red currant hybridizes readily with the common form and since this high type of resistance is controlled by a single dominant gene. Porte *et al.* (146) have reported *L. hirsutum* as highly resistant to wilt while also tolerant to common tomato mosaic (*tobacco virus 1* Johnson), and its value as a breeding stock is being explored.

In two notable cases Fusarium-wilt resistance has been combined with resistance to another important disease. Pritchard produced Marglobe from a cross between Marvel and Globe, the former being quite resistant to the nailhead spot (*Macrosporium tomato*) as well as to wilt. The two types of resistance are also combined in Marglobe (245). Shapavalov and Lesley (184) have recently introduced Riverside in which resistance to Fusarium wilt as well as to Verticillium wilt (*V. albo-atrum* R. & B.) occurs.

Leaf mold (*Cladosporium fulvum* Cke.). This disease is very

serious on glass-house tomatoes both in America and in Europe. All varieties of common tomato are more or less susceptible but red currant tomato is highly resistant. Sengbusch and Loschakowa-Hasenbusch (183) demonstrated that resistance in the latter was controlled by a single dominant gene. At about the same time Alexander (1) found a highly resistant off-type in Globe variety which was probably the result of a previous outcross with red currant tomato. From this, by a series of crosses and backcrosses, he developed and introduced a highly resistant variety of the pink-fruited Globe type under the name Globelle (2). Guba (71) has announced a red-fruited tomato resistant to leaf mold under the name Bay State.

Langford (98) has defined four physiologic races of the fungus, and mutation in pure cultures was found to occur. In addition to high resistance or immunity described for red currant, the latter is said to carry an independent dominant factor which in the absence of that for immunity governs an intermediate degree of resistance to the four races. An intermediate degree of resistance in Sterling Castle was found to be controlled by another dominant gene. The hypersensitive reaction of certain immunes was claimed to be due to a recessive lutescence factor in the homozygous condition.

In Langford's studies immunity to all races of the fungus was observed in red currant tomato. Soon after Globelle came into commercial use in northern Ohio, Alexander (3) noted typical leaf mold which rapidly increased in occurrence on this variety. This new strain of the fungus is distinct from all others previously described because it is rather highly pathogenic not only on Globelle but upon the red currant tomato. It is evident that the final success in the development of commercial leaf-mold-resistant tomatoes will rest upon the range of variability in the pathogen and the extent of corresponding resistant germ plasm in the host. Bond (25) has shown that the pathogen invades the stomata of highly resistant hosts readily though not so frequently, and this is also true of many non-infected species. The progress of the fungus is restricted sharply in the case of immune tomatoes and non-infective species but apparently is not necessarily accompanied by any visible cellular reaction.

Curly top. This virus disease is often extremely destructive to tomatoes in many sections of the United States west of the Continental Divide. A program for the development of resistant varieties

is under way under the leadership of Blood (23) in Utah. In this connection it is of importance to note that Virgin (211) in Idaho has found *Lycopersicon chilense* highly resistant to curly top, and the possibility of this character being transferred to common tomato is being explored. Lesley and Wallace (108) in southern California noted a tendency of tomato plants affected with curly top to show partial recovery from the disease. Wallace (243) noted a similar phenomenon with curly top on tobacco, in which he has demonstrated an acquired passive immunization which can be transferred to other plants through grafting. Since this is a phenomenon hitherto unreported in plants it has extremely important fundamental significance. Further study of this type of immunity in tomato and in plants generally may be expected.

BIBLIOGRAPHY

1. ALEXANDER, L. J. Leaf mold resistance in the tomato. Ohio Agr. Exp. Sta. Bul. 539. 1934.
2. ———. A new tomato variety resistant to leaf mold. Phytopath. 28: 1. 1938.
3. ———. A new strain of the tomato leaf mold fungus, *Cladosporium fulvum*. *Ibid.* 30: 1. 1940.
4. ANDERSON, M. E. Fusarium resistance in Wisconsin Hollander cabbage. Jour. Agr. Res. 47: 639–661. 1935.
5. ———, AND J. C. WALKER. Histological studies of Wisconsin Hollander and Wisconsin Ballhead cabbage in relation to resistance to yellows. *Ibid.* 50: 823–836. 1935.
6. ANGELL, H. R., J. C. WALKER, AND K. P. LINK. The relation of protocatechuic acid to disease resistance in the onion. Phytopath. 20: 431–439. 1930.
7. ATKINSON, G. F. Some diseases of cotton. Ala. Agr. Exp. Sta. Bul. 41. 1892.
8. BAILEY, R. M. Progress in breeding cucumbers resistant to scab (*Cladosporium cucumerinum*). Amer. Soc. Hort. Sci. Proc. 36(1938): 645–646. 1939.
9. ———, AND I. M. BURGESS. Breeding cucumbers resistant to scab. *Ibid.* 32(1934): 474–476. 1935.
10. BARRUS, M. F. Variation of varieties of beans in their susceptibility to anthracnose. Phytopath. 1: 190–195. 1911.
11. ———. Anthracnose-resistant red kidney bean. *Ibid.* 5: 301–311. 1915.
12. ———. Varietal susceptibility of beans to strains of *Colletotrichum lindemuthianum* (Sacc. & Magn.) B. & C. *Ibid.* 8: 589–614. 1918.
13. BENNETT, L. S. Studies on the inheritance of resistance to wilt (*Fusarium niveum*) in watermelon. Jour. Agr. Res. 53: 295–306. 1936.
14. BERG, A. Tomato late blight and its relation to late blight of potatoes. W. Va. Agr. Exp. Sta. Bul. 205. 1926.
15. BEVER, W. M. Physiologic specialization in *Puccinia glumarum* in the United States. Phytopath. 24: 686–688. 1934.
16. BIFFEN, R. H. Mendel's laws of inheritance and wheat breeding. Jour. Agr. Sci. 1: 4–48. 1905.
17. ———. Studies in inheritance of disease resistance II *Ibid* 4:

18. BLACK, W. Studies on the inheritance of resistance to wart disease (*Synchytrium endobioticum* (Schilb.) Perc.) in potatoes. Jour. Genet. **30**: 127–146. 1935.
19. BLANK, L. M. Uniformity in pathogenicity and cultural behavior among strains of the cabbage-yellows organism. Jour. Agr. Res. **48**: 401–409. 1934.
20. ———. Fusarium resistance in Wisconsin All Seasons cabbage. *Ibid.* **55**: 497–510. 1937.
21. ———, AND J. C. WALKER. Inheritance of Fusarium resistance in brussels sprouts and kohlrabi. *Ibid.* **46**: 1015–1022. 1933.
22. BLINN, P. K. A rust resisting cantaloupe. Col. Agr. Exp. Sta. Bul. 104. 1905.
23. BLOOD, H. L. A method of measuring the relative resistance of varieties of tomato and bean to curly top. Utah Acad. Sci. Proc. **15** (1937–8): 21–24. 1938.
24. BOHN, G. W., AND C. M. TUCKER. Studies on Fusarium wilt of the tomato. I. Immunity in *Lycopersicon pimpinellifolium* Mill. and its inheritance in hybrids. Mo. Agr. Exp. Sta. Res. Bul. 311. 1940.
25. BOND, T. E. T. Infection experiments with *Cladosporium fulvum* Cooke and related species. Ann. Appl. Biol. **25**: 277–307. 1938.
26. BONDE, R., F. J. STEVENSON, AND C. F. CLARK. Resistance of certain potato varieties and seedling progenies to late blight in the tubers. Phytopath. **30**: 733–748. 1940.
27. BÖNING, K. Ueber die Empfänglichkeit von *Phaseolus vulgaris* für *Colletotrichum lindemuthianum* im Lichte der Rassenbildung des Krankheitserregers. Forsch. Geb. Pflanzenk. u. Immunität im Pflanzenreich **2**: 4–18. 1926.
28. ———, AND F. WALLNER. Beobachtungen und Versuche zur Frage der Widerstandsfähigkeit der Kartoffelsorten gegen Schorf. Prakt. Bl. Pflanzenbau Pflanzenschutz. **15**: 268–279. 1938.
29. BORDUKOVA, M. W. MME. Ueber die Bestimmung der Widerstandsfähigkeit der Kartoffeln gegen die Phytophthora. Obst. u. Gemüsebau Wirtsch. **9**: 29–32. 1937.
30. BUDDE, A. Ueber Rassenbildung parasitischer Pilze unter besonderer Berücksichtigung von *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Cav. in Deutschland. Forsch. Geb. Pflanzenk. u. Immunität im Pflanzenreich **5**: 115–147. 1928.
31. BUKASOV, S. M. The problems of potato breeding. Amer. Potato Jour. **13**: 235–252. 1936.
32. BURKHOLDER, W. H. The production of an anthracnose-resistant White Marrow bean. Phytopath. **8**: 353–359. 1918.
33. ———. The gamma strain of *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C. *Ibid.* **13**: 316–323. 1923.
34. ———. Varietal susceptibility among beans to the bacterial blight. *Ibid.* **14**: 1–7. 1924.
35. ———, AND K. ZALESKI. Varietal susceptibility of beans to an American and a European strain of *Phytomonas medicaginis* var. *phaseolicola*, and a comparison of the strains in culture. *Ibid.* **22**: 85–94. 1932.
36. BUTLER, E. J. The bearing of Mendelism on the susceptibility of wheat to rust. Jour. Agr. Sci. **1**: 361–363. 1905.
37. CARTWRIGHT, K. On the nature of the resistance of the potato to the wart disease. Ann. Bot. **40**: 391–395. 1926.
38. CHRISTENSEN, C. J. Tiltraekning af kaalroeformer med saerlig modstandsevne mod kaalbroksvamp. Tidsskr. for. Planteavl **24**: 68–82. 1917.
39. COBB, N. A. Contributions to an economic knowledge of the Australian rusts (Uredineae). Agr. Gaz. N.S. Wales **1**: 185–214, 1890; **3**: 44–68, 181–212, 1892.

40. Cochran, F. D. Breeding cucumbers for resistance to downy mildew. Amer. Soc. Hort. Sci. Proc. 35 (1937): 541–543. 1938.

41. Collins, E. J. The problem of immunity to wart disease (*Synchytrium endobioticum* (Schilb.) Perc.) in the potato. Ann. Bot. **49**: 479–491. 1935.

42. Cook, H. T. Powdery mildew disease of snap beans. Va. Truck Exp. Sta. Bul. 74: 931–940. 1931.

43. ———. Watermelon wilt and resistant varieties for its control. Va. Truck Exp. Sta. Bul. 97: 1513–1526. 1937.

44. Clark, C. F., W. P. Raleigh, and F. J. Stevenson. Breeding for resistance to common scab in the potato. Amer. Potato Jour. **13**: 256–259. 1936.

45. ———, F. J. Stevenson, and L. A. Schaal. The inheritance of scab resistance in certain crosses and selfed lines of potatoes. Phytopath. **28**: 878–890. 1938.

46. ———, W. Stuart, and F. J. Stevenson. The Katahdin and Chippewa potatoes. U. S. Dept. Agr. Cir. 276. 1933.

47. Dana, B. F. Resistance and susceptibility to curly top in varieties of squash, *Cucurbita maxima*. Phytopath. **28**: 649–656. 1938.

48. Darling, H. M. A study of scab resistance in the potato. Jour. Agr. Res. **54**: 305–317. 1937.

49. DeBary, A. Untersuchungen über die Brandpilze und die durch sie verursachten Krankheiten der Pflanzen mit Rücksicht auf das Getreide und andere Nutzpflanzen. 1853.

50. DeBruyn, Helena L. G. Onderzoekingen over enkele Actinomyceten, welke Aardappelschurft verwekken. Tijdschr. over Plantenziekten **45**: 133–156. 1939.

51. Delwiche, E. J., F. L. Musbach, Emil Truog, J. C. Walker, and H. F. Wilson. Canning peas in Wisconsin. Wis. Agr. Exp. Sta. Bul. 444. 1939.

52. Doolittle, S. P. Comparative susceptibility of European and American varieties of cucumbers to bacterial wilt. Phytopath. **12**: 143–146. 1922.

53. ———, F. S. Beecher, and W. S. Porte. A hybrid cucumber resistant to bacterial wilt. *Ibid.* **29**: 996–998. 1939.

54. Doornkat-Koolman, H. ten. Die Brennfleckenkrankheit der Gartenbohne im Lichte Vererbung. Versuche zur Immunitszüchtung bei *Phaseolus vulgaris* gegenüber *Colletotrichum lindemuthianum* (Sacc. & Magn.) und seinen Biotypen. Forsch. Geb. Pflanzenk. u. Immunität in Pflanzenreich **4**: 112–237. 1927.

55. Dundas, B. Inheritance of resistance to powdery mildew in beans. Hilgardia **10**: 243–253. 1936.

56. ———. Inheritance of resistance to powdery mildew in runner beans (*Phaseolus coccineus*), tepary beans (*P. acutifolius*), yard long beans (*Vigna sesquipedalis*) and cowpeas (*Vigna sinensis*). Phytopath. **29**: 824. 1939.

57. ———, and G. W. Scott. Physiologic strains of bean rust. *Ibid.* **29**: 820–821. 1939.

58. Edgerton, C. W., and C. C. Moreland. Tests of the wilt resistance of different tomato varieties. La. Agr. Exp. Sta. Bul. 184. 1921.

59. Elmer, O. H. A mosaic resistant variety of cucumbers. Phytopath. **17**: 48. 1927.

60. Ericksson, J. Ueber die Spezialisierung des Parasitismus bei den Getreiderostpilzen. Ber. Deut. Bot. Ges. **12**: 292–331. 1894.

61. Essary, S. H. Notes on tomato diseases with results of selection for resistance. Tenn. Agr. Exp. Sta. Bul. 95. 1912.

62. Evans, I. B. Pole. South African cereal rusts, with observations on the problem of breeding rust-resistant wheats. Jour. Agr. Sci. **4**: 95–104. 1911.

63. Evans, R. I. Cytological studies on the parasitic relationship of *Urocystis cepulae* to the onion. Amer. Jour. Bot. **20**: 255–268. 1933.
64. ———. Cytological studies on the parasitic relationship of *Urocystis cepulae* to *Allium fistulosum*. *Ibid.* **24**: 214–218. 1937.
65. Felix, E. L. Disease resistance in *Allium fistulosum* L. Phytopath. **23**: 109–110. 1933.
66. Fromme, F. D., and S. A. Wingard. Bean rust: its control through the use of resistant varieties. Va. Agr. Exp. Sta. Bul. 220. 1918.
67. ———, and ———. Varietal susceptibility of beans to rust. Jour. Agr. Res. **21**: 385–404. 1921.
68. Gibson, C. M. Notes on infection experiments with various Uredineae. New Phytol. **3**: 184–191. 1904.
69. Giddings, N. J., and A. Berg. A comparison of the late blights of tomato and potato. A preliminary report. Phytopath. **9**: 209–210. 1919.
70. Glynne, M. D. Infectivity of summer sporangia of potato wart disease in incipient infections on varieties immune in the field. Nature **134**: 253. 1934.
71. Guba, E. F. A red forcing tomato resistant to *Cladosporium* leaf mold. Phytopath. **29**: 9. 1939.
72. Harter, L. L., C. F. Andrus, and W. J. Zaumeyer. Studies on bean rust caused by *Uromyces phaseoli typica*. Jour. Agr. Res. **50**: 737–759. 1935.
73. ———, and J. L. Weimer. A monographic study of sweet potato diseases and their control. U. S. Dept. Agr. Tech. Bul. 99. 1929.
74. ———, and W. A. Whitney. The comparative susceptibility of sweet potato varieties to stem rot. Jour. Agr. Res. **34**: 915–919. 1927.
75. Hawkins, L. A., and R. B. Harvey. Physiological study of the parasitism of *Pythum debaryanum* Hesse on the potato tuber. *Ibid.* **18**: 275–297. 1919.
76. Haymaker, H. H. Pathogenicity of two strains of the tomato-wilt fungus, *Fusarium lycopersici* Sacc. *Ibid.* **36**: 675–695. 1928.
77. Henderson, W. J. Yellow dwarf a virus disease of onions, and its control. Iowa Agr. Exp. Sta. Res. Bul. 188: 211–255. 1935.
78. Hoggan, Ismé A. Some viruses affecting spinach and certain aspects of insect transmission. Phytopath. **23**: 446–474. 1933.
79. Holmes, F. O. Inheritance of resistance to tobacco-mosaic disease in the pepper. *Ibid.* **27**: 637–642. 1937.
80. Huber, G. A., and C. D. Schwartze. Resistance in the red raspberry to the mosaic vector *Amphorophora rubi* Kalt. Jour. Agr. Res. **57**: 623–633. 1938.
81. Huelsen, W. A. Wilt-resistant tomato varieties released by the Illinois Station. Ill. Agr. Exp. Sta. Cir. 490. 1939.
82. Jagger, I. C. Brown blight of lettuce. Phytopath. **30**: 53–64. 1940.
83. ———, and G. W. Scott. Development of powdery mildew resistant cantaloup No. 45. U. S. Dept. Agr. Cir. 441. 1937.
84. ———, and T. W. Whitaker. The inheritance of immunity from mildew (*Bremia lactucae*) in lettuce. Phytopath. **30**: 427–433. 1940.
85. ———, ———, and D. R. Porter. Inheritance in *Cucumis melo* of resistance to powdery mildew (*Erysiphe cichoracearum*). *Ibid.* **28**: 671. 1938.
86. ———, ———, and ———. A new biologic form of powdery mildew on muskmelons in the Imperial Valley of California. Plant Dis. Rptr. **22**: 275–276. 1938.
87. Johnson, B. Specificity to penetration of the epidermis of a plant by the hyphae of a pathogenic fungus. Amer. Jour. Bot. **19**: 12–31. 1932.

88. JOHNSON, J. Relation of water-soaked tissues to infection by *Bacterium angulatum* and *Bact. tabacum* and other organisms. Jour. Agr. Res. 55: 599–618. 1937.
89. JONES, H. A. Vegetable breeding at the University of California. Amer. Soc. Hort. Sci. Proc. 29(1932): 572–581. 1933.
90. JONES, L. K., C. L. VINCENT, AND E. F. BURK. The resistance of progeny of Katahdin potatoes to viroses. Jour. Agr. Res. 60: 631–644. 1940.
91. JONES, L. R. Disease resistance of potatoes. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 87. 1905.
92. ————, AND J. C. GILMAN. The control of cabbage yellows through disease resistance. Wis. Agr. Exp. Sta. Res. Bul. 38. 1915.
93. ————, J. C. WALKER, AND W. B. TISDALE. Fusarium resistant cabbage. Wis. Agr. Exp. Sta. Res. Bul. 48. 1920.
94. KENDRICK, J. B. Kale yellows in California, caused by *Fusarium conglutinans* Wollenw. Hilgardia 5: 1–15. 1930.
95. KÖHLER, E. Ueber das Verhalten von *Synchytrium endobioticum* auf anfälligen und widerstandsfahigen Kartoffelsorten. Biol. Reichsanst. Land u. Forstw. Arb. 19: 263–285. 1931.
96. KOTTE, W. Zur Kenntnis der "Fettfleckenkrankheit" der Bohne. Ztschr. Pflanzenkr. 41: 12–19. 1931.
97. KÜHN, J. G. Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung. 1858.
98. LANGFORD, A. N. The parasitism of *Cladosporium fulvum* Cooke and the genetics of resistance to it. Canad. Jour. Res. (Sect. C) 15: 108–128. 1937.
99. LAURITZEN, J. I. A strain of Yellow Jersey sweet potato resistant to surface rot (*Fusarium oxysporum* W. & C.). Jour. Agr. Res. 33: 1091–1094. 1926.
100. LAYTON, D. V. The parasitism of *Colletotrichum lagenarium* (Pass.) Ell. and Halst. Iowa Agr. Exp. Sta. Res. Bul. 223: 37–67. 1937.
101. LEACH, J. G. The parasitism of *Colletotrichum lindemuthianum*. Minn. Agr. Exp. Sta. Tech. Bul. 14. 1923.
102. ————, AND T. M. CURRENCE. Fusarium wilt of muskmelons in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 129. 1938.
103. ————, P. DECKER, AND HANNAH BECKER. Pathogenic races of *Actinomyces scabies* in relation to scab resistance. Phytopath. 29: 204–209. 1939.
104. ————, F. A. KRANTZ, P. DECKER, AND H. MATTSON. The measurement and inheritance of scab resistance in selfed and hybrid progenies of potatoes. Jour. Agr. Res. 56: 843–853. 1938.
105. LEHMANN, H. Ein weiterer Beitrag zum Problem der physiologischen Spezialisierung von *Phytophthora infestans* deBary, dem Erreger der Kartoffelkrautfäule. Phytopath. Ztschr. 11: 121–154. 1938.
106. LEHMANN, H. Das heutige Ausgangsmaterial für die Züchtung *Phytophthora*-widerstandsfähiger Kartoffeln. Züchter 9: 29–35. 1937.
107. LEMMERZAHL, J. Zur Methodik der Krebsprüfung von Kartoffelstämmen. *Ibid.* 3: 138–152. 1931.
108. LESLEY, J. W., AND J. M. WALLACE. Acquired tolerance to curly top in the tomato. Phytopath. 28: 548–553. 1938.
109. LINFORD, M. B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.
110. ————. Studies of pathogenesis and resistance in pea wilt caused by *Fusarium orthoceras* var. *pisi*. Phytopath. 21: 797–826. 1931.
111. ————. Wound inoculation in relation to resistance in the Fusarium wilt of peas. *Ibid.* 21: 827–833. 1931.
112. LINK, K. P., H. R. ANGELL, AND J. C. WALKER. The isolation of pro-

tocatechuic acid from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. **81**: 369–375. 1929.

113. ———, A. D. Dickson, and J. C. Walker. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. *Ibid.* **84**: 719–725. 1929.

114. ———, and J. C. Walker. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *Ibid.* 100: 379–383. 1933.

115. Lunden, A. P., and I. Jørstad. Investigations on the inheritance of immunity to wart disease (*Synchytrium endobioticum* (Schilb.) Perc.) in the potato. Jour. Genet. **29**: 375–385. 1934.

116. MacDonald, J. A. Plant pathology. Scot. Jour. Agr. **18**: 164–167. 1935.

117. Malabanan, D. B. Anthracnose of pepper. Philippine Agr. **14**: 491–502. 1926.

118. Manns, T. F., and J. F. Adams. Department of plant pathology. Del. Agr. Exp. Sta. Bul. 152 (Ann. Rpt. 1927): 40–51. 1927.

119. Mayer, A. E. Ueber die Mosaikkrankheit des Tabaks. Landw. Ver. Sta. **32**: 451–467. 1886.

120. Melchers, L. E., and J. H. Parker. Another strain of *Puccinia graminis*. Kans. Agr. Exp. Sta. Cir. 68. 1918.

121. McRostie, G. P. Inheritance of disease resistance in the common bean. Jour. Amer. Soc. Agron. **13**: 15–32. 1921.

122. Mills, W. R. Adaptive parasitism of *Phytophthora infestans*. Phytopath. **30**: 17. 1940.

123. ———. *Phytophthora infestans* on tomato. *Ibid.* **30**: 830–839. 1940.

124. Moore, W. D. Powdery mildew (*Erysiphe polygoni*) on garden snap beans. *Ibid.* **26**: 1135–1141. 1936.

125. Muller, H. R. A. Physiologic forms of *Colletotrichum lindemuthianum* (Sacc. et Mag.) Bri. et Cav. in the Netherlands. *Ibid.* **16**: 369. 1926.

126. Müller, K. O. Ueber die *Phytophthora*-resistenz der Kartoffel und ihre Vererbung. (Zugleich ein Beitrag zur Frage der Polyploidie bei der Kartoffel). Ang. Bot. **12**: 299–324. 1930.

127. ———. Die Variabilität der Virulenz und der biologischen Spezialization bei dem Erreger der Kartoffelkrautfäule, *Phytophthora infestans*. Naturwissen. **24**: 552–557. 1936.

128. Murphy, D. M. A great northern bean resistant to curly-top and common bean-mosaic viruses. Phytopath. **30**: 779–784. 1940.

129. ———, and W. H. Pierce. Common mosaic of garden pea, *Pisum sativum*. *Ibid.* **27**: 710–721. 1936.

130. ———, and ———. A mosaic resistant small red bean. *Ibid.* **28**: 270–273. 1938.

131. Nelson, R., G. H. Coons, and L. C. Cochran. The Fusarium yellows disease of celery (*Apium graveolens* L. var. *dulce* D. C.). Mich. Agr. Exp. Sta. Tech. Bul. 155. 1937.

132. Nilsson-Ehle, H. Resistenz gegen Gelbrost beim Weisen. Kreuzungsuntersuchungen an Hafer und Weisen. II. Lund's Univ. Årsskr. n. f. afd. 2, 7: 57–82. 1911.

133. Norton, J. B. Methods used in breeding asparagus for rust resistance. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 263. 1913.

134. ———. Washington asparagus: information and suggestions for growers of new pedigreed rust-resistant strains. U. S. Dept. Agr. Cotton, Truck, and Forage Crop Dis. Cir. 7. 1919.

135. Ogilvie, L., and C. J. Hickman. Progress report on vegetable diseases. IX. Bristol Univ., Agr. and Hort. Res. Sta. Ann. Rpt. 1937: 96–109. 1938.

136. OLSSON, P. A. Svalöfs Majrova. En Rovsort, speciellt för odlingsområden där klumprotsjukan härjar. Sevriges Utsädesför. Tidskr. **48**: 471–476. 1939.
137. ORTON, W. A. A study of disease resistance in watermelons. Science **25**: 288. 1907.
138. ———. The development of farm crops resistant to disease. U. S. Dept. Agr. Yearbook 1908: 453–464. 1909.
139. PARKER, M. C. Inheritance of resistance to the common mosaic virus in the bean. Jour. Agr. Res. **52**: 895–915. 1936.
140. PARRIS, G. K. The reactions of introduced bean varieties to rust (*Uromyces phaseoli typica*) in Hawaii. Plant Dis. Rptr. **22**: 424–428. 1938.
141. PEUSER, H. Fortgesetzte Untersuchungen über das Verkommen biologischer Rassen von *Colletotrichum lindemuth.* (Sacc. et Mgn.) Bri. et Cav. Phytopath. Ztschr. **4**: 83–112. 1931.
142. PIERCE, W. H. Viruses of the bean. Phytopath. **24**: 87–115. 1934.
143. ———. Resistance to common bean mosaic in the Great Northern field bean. Jour. Agr. Res. **49**: 183–188. 1934.
144. ———. The inheritance of resistance to the common bean mosaic in field and garden beans. Phytopath. **25**: 875–883. 1935.
145. ———, AND J. C. WALKER. The development of mosaic resistant Refugee beans. Canner **77** (26): 7–9. 1933.
146. PORTE, W. S., S. P. DOOLITTLE, AND F. L. WELLMAN. Hybridization of a mosaic-tolerant, wilt-resistant *Lycopersicon hirsutum* with *Lycopersicon esculentum*. Phytopath. **29**: 757–759. 1939.
147. PORTER, D. R. Breeding high-quality wilt-resistant watermelons. Calif. Agr. Exp. Sta. Bul. 614. 1937.
148. ———, AND H. A. JONES. Resistance of some of the cultivated species of *Allium* to pink root (*Phoma terrestris*). Phytopath. **23**: 290–298. 1933.
149. ———, AND I. E. MELHUS. The pathogencity of *Fusarium niveum* E.F.S. and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad.). Iowa Agr. Exp. Sta. Res. Bul. 149: 124–184. 1932.
150. PORTER, R. H. The reaction of cucumbers to types of mosaic. Iowa State Col. Jour. Sci. **6**: 95–120. 1931.
151. PRÉVOST, B. Mémoire sur la cause immédiate de la carie ou Charbon des blés et de plusieurs autres maladies des plantes, et sur les préservatifs de la carie. 1807. [English translation by G. W. Keitt, Phytopathological Classics **6**, 1939.]
152. PRICE, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus. Amer. Jour. Bot. **17**: 694–702. 1930.
153. PRITCHARD, F. J. Development of wilt-resistant tomatoes. U. S. Dept. Agr. Bul. 1015. 1922.
154. RANDS, R. D., AND W. BROTHERTON. Bean varietal tests for disease resistance. Jour. Agr. Res. **31**: 101–154. 1925.
155. REDDICK, D. Blight immune, drought tolerant potatoes. Amer. Potato Jour. **14**: 205–210. 1937.
156. ———. Scab immunity. *Ibid.* **16**: 71–74, 76. 1939.
157. ———, AND W. CROSIER. Biological specialization in *Phytophthora infestans*. *Ibid.* **10**: 129–134. 1933.
158. ———, AND W. MILLS. Building up virulence in *Phytophthora infestans*. *Ibid.* **15**: 29–34. 1938.
159. ———, AND ———. Blight immune versus blight resistant potatoes. *Ibid.* **16**: 220–224. 1939.
160. ———, AND V. B. STEWART. Varieties of beans susceptible to mosaic. Phytopath. **8**: 530–534. 1918.
161. ———, AND ———. Additional varieties of beans susceptible to mosaic. *Ibid.* **9**: 149–152. 1919.

162. RIEMAN, G. H. Genetic factors for pigmentation in the onion and their relation to disease resistance. Jour. Agr. Res. 42: 251–278. 1931.
163. ROCHLIN, E. Zur Frage der Widerstandsfähigkeit der Cruciferen gegen die Kohlhernie (*Plasmodiophora brassicae* Wor.). Phytopath. Ztschr. 5: 381–406. 1932.
164. ROQUE, A. New cucumbers strains resistant to downy mildew are high yielders. Puerto Rico Agr. Exp. Sta. Ann. Rpt. 1938–39: 61. 1939.
165. ———, AND J. ADSUAR. New cucumber varieties resistant to the downy mildew. *Ibid.* 1937–38: 45–46. 1939.
166. ———, AND ———. The development of new varieties of eggplant resistant to bacterial wilt. *Ibid.* 1937–38: 44–45. 1939.
167. ———, AND ———. Pepper mosaic, a serious disease in Puerto Rico, may be controlled by breeding resistant varieties. *Ibid.* 1938–39: 62. 1939.
168. ROSENBAUM, J., AND C. E. SANDO. Correlation between size of the fruit and the resistance of the tomato skin to puncture and its relation to infection with *Macrosporium tomato* Cooke. Amer. Jour. Bot. 7: 78–82. 1920.
169. RYKER, T. C. Fusarium yellows of celery. Phytopath. 25: 578–600. 1935.
170. SALAMAN, R. N. Potato variety production: a new departure. Gard. Chron. 102: 326–327. 1937.
171. ———, AND J. W. LESLEY. Genetic studies in potatoes: the inheritance of immunity to wart disease. Jour. Genet. 13: 177–186. 1923.
172. SCHICK, R. Ueber das Verhalten von *Solanum demissum*, *Solanum tuberosum* und ihrer Bastarden gegenüber verschiedenen Herkunfter von *Phytophthora infestans*. Züchter 4: 233–237. 1932.
173. ———, AND H. LEHMANN. Zur physiologischen Specialisierung von *Phytophthora infestans* de Bary. Zugleich ein Beitrag zur Methodik der Züchtung kräutfaulewiderstandsfähiger Kartoffeln. *Ibid.* 8: 34–46. 1936.
174. ———, AND P. SCHAPER. Das Verhalten von verschiedenen Formen von *Solanum demissum* gegenüber 4 verschiedenen Linien der *Phytophthora infestans*. *Ibid.* 8: 102–104. 1936.
175. SCHLUMBERGER, O. Kartoffelsorten—Prüfung auf Schorfwiderstandsfähigkeit. Mitt. Landw. 52: 52–53, 1937; 53: 99, 1938.
176. SCHREIBER, F. Resistenzüchtung bei *Phaseolus vulgaris*. Phytopath. Ztschr. 4: 415–454. 1932.
177. ———. Resistenz-Züchtung bei Buschbonen. Kühn-Archiv. 38: 287–292. 1933.
178. SCHULTZ, E. S., C. F. CLARK, R. BONDE, W. P. RALEIGH, AND F. J. STEVENSON. Resistance of potato to mosaic and other virus diseases. Phytopath. 24: 116–132. 1934.
179. ———, ———, W. P. RALEIGH, F. J. STEVENSON, R. BONDE, AND J. H. BEAUMONT. Recent developments in potato breeding for resistance to virus diseases. *Ibid.* 27: 190–197. 1937.
180. SCHULTZ, H. Untersuchungen über das Verhalten von Gurkensorten bei künstlicher Infection mit *Cladosporium cucumerinum* Ell. et Arth. sowie einige morphologische Eigenschaft des Pilzes. Gartenbauwiss. 13: 505–618. 1939.
181. ———, AND K. RÖDER. Die Anfälligkeit verschiedener Varietäten und Sorten von Salat (*Lactuca sativa* L. und *Lactuca scariola* L.) gegen den falschen Meltau (*Bremia lactucae* Regel). Züchter 10: 185–194. 1938.
182. ———, AND ———. Freilandbeobachtungen über die Anfälligkeit von Gurken (*Cucumis sativus* L.) gegen Krätze, Blattbraune und Mehltau. Gartenbäuwiss. 13: 169–183. 1939.
183. SENGBUSCH, R. V., AND N. LOSCHAKOWA-HASENBUSCH. Immunitäts-

zuchtung bei Tomaten. Vorläufige über die Züchtung gegen die Braunfleckenkrankheit (*Cladosporium fulvum* Cooke.) resistenter Sorten. Züchter 4: 257–264. 1932.

184. SHAPOVALOV, M., AND J. W. LESLEY. Wilt resistance of the Riverside variety of tomato to both Fusarium and Verticillium wilts. Phytopath. 30: 760–768. 1940.

185. SIDOROV, F. F. Züchtung phytophthorawiderstandfähiger Kartoffelsorten. *Ibid.* 27: 211–241. 1937.

186. SILAYAN, H. S. Philippine Dept. Agr. and Nat. Resources. Bur. Plant Indus. Ann. Rpt. 1937: 21. 1938.

187. SLEETH, B. *Fusarium niveum,* the cause of watermelon wilt. W. Va. Agr. Exp. Sta. Bul. 257. 1934.

188. SMITH, E. F. The fungous infestation of agricultural soils in the United States. Sci. Amer. Sup. 48: 19981–19982. 1899.

189. SMITH, F. L., AND W. B. HEWITT. Varietal susceptibility to common bean mosaic and transmission through seed. Calif. Agr. Exp. Sta. Bul. 621. 1938.

190. SMITH, L. B. Breeding mosaic resistant spinach and notes on malnutrition. Va. Truck Exp. Sta. Bul. 135: 31–32. 1920.

191. SMITH, ROSE, AND J. C. WALKER. A cytological study of cabbage plants in strains susceptible or resistant to yellows. Jour. Agr. Res. 41: 17–35. 1930.

192. SNELL, JOHN. Ormskirk potato trials. Annual report, 1918. Jour. Bd. Agr. [Gt. Britain] Suppl. 18: 68–102. 1919.

193. SNYDER, W. C. Variability of the pea-wilt organism, *Fusarium orthoceras* var. *pisi.* Jour. Agr. Res. 47: 65–88. 1933.

194. ————, AND B. A. RUDOLPH. Verticillium wilt of pepper, *Capsicum annum.* Phytopath. 29: 359–362. 1939.

195. ————, AND J. C. WALKER. Fusarium near-wilt of pea. Zentbl. Bakt. [etc.] Abt. II, 41: 355–378. 1935.

196. STAKMAN, E. C. The genetics of pathogenic organisms. Publ. Amer. Assoc. Adv. Sci. 12. 1940.

197. ————, AND F. J. PIEMEISEL. A new strain of *Puccinia graminis.* Phytopath. 7: 73. 1917.

198. STAPP, C., AND H. HAHNE. Zur Frage der Resistenz von Buschbohnensorten gegen den Erreger der Fettfleckenkrankheit *Pseudomonas medicaginis* var. *phaseolicola* Burkh. Ang. Bot. 18: 249–262. 1936.

199. STEVENSON, F. J., AND C. F. CLARK. The Sebago potato, a new variety resistant to late blight. U. S. Dept. Agr. Cir. 503. 1938.

200. ————, E. S. SCHULTZ, AND C. F. CLARK. Inheritance of immunity from virus X (latent mosaic) in the potato. Phytopath. 29: 362–365. 1939.

201. ————, ————, ————, L. CASH, AND R. BONDE. Breeding for resistance to late blight in the potato. *Ibid.* 27: 1059–1070. 1937.

202. ————, ————, ————, W. P. RALEIGH, L. C. CASH, AND R. BONDE. Breeding for resistance to late blight in the potato. Amer. Potato Jour. 13: 205–218. 1936.

203. STRAIB, W. Untersuchungen zur Genetik der Gelbrostresistenz des Weizens. Phytopath. Ztschr. 7: 427–477. 1934.

204. ————. Die Untersuchungsergebnisse zur Frage der biologischen Specialisierung des Gelbrostes (*Puccinia glumarum*) und ihre Bedeutung für die Pflanzenzüchtung. Züchter 5: 118–129. 1937.

205. STRONG, (MRS.) M. C. A new Fusarium-wilt-resistant tomato. Mich. Agr. Exp. Sta. Quart. Bul. 21: 164–169. 1939.

206. STUART, WM. Disease resistance of potatoes. Vt. Agr. Exp. Sta. Bul. 122: 107–136. 1906.

207. STUBBS, M. W. Certain viroses of the garden pea, *Pisum sativum*. Phytopath. **27**: 242–266. 1937.
208. TIMS, E. C. On the nature of resistance to cabbage yellows. Jour. Agr. Res. **32**: 183–199. 1926.
209. TOWNSEND, G. R. Reactions of varieties of snap beans to rust. Plant Dis. Rptr. **22**: 2–4. 1938.
210. TULASNE, L. R., AND CHARLES. Mémoire sur les Ustilaginées comparées aux Urédinées. Ann. Sci. Nat., III. **7**: 12–127. 1847.
211. VIRGIN, W. J. The Chilean tomato, *Lycopersicon chilense*, found resistant to curly top. Phytopath. **30**: 280. 1940.
212. ————, AND J. C. WALKER. Relation of temperature and moisture to near-wilt of pea. Jour. Agr. Res. **59**: 591–600. 1939.
213. ————, AND ————. Relation of near-wilt fungus to the pea plant. *Ibid*. **60**: 241–248. 1940.
214. WADE, B. L. The inheritance of Fusarium wilt resistance in canning peas. Wis. Agr. Exp. Sta. Res. Bul. 97. 1929.
215. ————, AND W. J. ZAUMEYER. Varietal reaction of pea to a virus from alsike clover. Phytopath. **28**: 505–511. 1938.
216. ————, AND ————. U. S. No. 5 Refugee, a new mosaic-resistant Refugee bean. U. S. Dept. Agr. Cir. 500. 1938.
217. ————, AND ————. Genetic studies of resistance to alfalfa mosaic virus and of stringiness in *Phaseolus vulgaris*. Jour. Amer. Soc. Agron. **32**: 127–134. 1940.
218. ————, ————, AND L. L. HARTER. Variety studies in relation to Fusarium wilt of peas. U. S. Dept. Agr. Cir. 473. 1938.
219. WALKER, J. C. Onion smudge. Jour. Agr. Res. **20**: 685–721. 1921.
220. ————. Disease resistance to onion smudge. *Ibid*. **24**: 1019–1040. 1923.
221. ————. Studies upon the inheritance of Fusarium resistance in cabbage. Phytopath. **16**: 87. 1926.
222. ————. Resistance to Fusarium wilt in garden, canning and field peas. Wis. Agr. Exp. Sta. Res. Bul. 107. 1931.
223. ————. Inheritance of Fusarium resistance in cabbage. Jour. Agr. Res. **40**: 721–745. 1930.
224. ————. Yellows-resistant lines of Jersey Wakefield cabbage. *Ibid*. **46**: 639–648. 1933.
225. ————. A study of resistance to Fusarium wilt in Alaska peas. Amer. Jour. Bot. **22**: 849–857. 1935.
226. ————. Resistance to club root in Brassica. Phytopath. **26**: 112. 1936.
227. ————. Diseases of cabbage and related plants. U. S. Dept. Agr. Farmers' Bul. 1439. 1927. (Rev. 1938.)
228. ————. Resistance to clubroot in varieties of turnip and rutabaga. Jour. Agr. Res. **59**: 815–827. 1939.
229. ————. Disease resistant pea varieties. Canner **88**(12, pt. 2): 89. 1939.
230. ————, J. F. ADAMS, AND W. H. PIERCE. Results of planting tests with Wisconsin Refugee and Idaho Refugee beans. Canner **80**(12): 7–8, 22. 1935.
231. ————, AND L. M. BLANK. Fusarium-resistant Danish Ballhead cabbage. Jour. Agr. Res. **49**: 983–989. 1934.
232. ————, AND R. H. LARSON. Yellow dwarf of potato in Wisconsin. *Ibid*. **59**: 259–280. 1939.
233. ————, ————, AND A. R. ALBERT. Studies of resistance to potato scab in Wisconsin. Amer. Potato Jour. **15**: 246–252. 1938.
234. ————, AND C. C. LINDEGREN. Further studies on the relation of onion scale pigmentation to disease resistance. Jour. Agr. Res. **29**: 507–514. 1924.
235. ————, ————, AND FREDA BACHMAN. Further studies on the

toxicity of juice extracted from succulent onion scales. *Ibid.* **30**: 175–187. 1925.
236. ———, AND K. P. LINK. Toxicity of phenolic compounds to certain onion bulb parasites. Bot. Gaz. **99**: 468–484. 1935.
237. ———, ———, AND S. MORELL. Find no relation between clubroot resistance and volatile oil content of plants. Wis. Agr. Exp. Sta. Bul. 435: 102–103. 1936.
238. ———, J. MONTEITH, AND F. L. WELLMAN. Development of three midseason varieties of cabbage resistant to yellows (*Fusarium conglutinans* Woll.). Jour. Agr. Res. **35**: 785–809. 1927.
239. ———, AND R. SMITH. Effect of environmental factors upon the resistance of cabbage to yellows. *Ibid.* **41**: 1–15. 1930.
240. ———, AND W. C. SNYDER. Pea wilt and root rots. Wis. Agr. Exp. Sta. Bul. 424. 1933.
241. ———, AND F. L. WELLMAN. A survey of the resistance of subspecies of *Brassica oleracea* to yellows (*Fusarium conglutinans*). Jour. Agr. Res. **37**: 233–241. 1928.
242. WALKER, M. N. A wilt-resistant watermelon for Florida. Fla. Agr. Exp. Sta. Bul. 288. 1936.
243. WALLACE, J. W. Evidence of passive immunization of tobacco, Nicotiana tabacum, from the virus of curly top. Phytopath. **30**: 673–679. 1940.
244. WARD, H. M. On the relations between host and parasite in the bromes and their brown rust, *P. dispersa* Erikss. Ann. Bot. **16**: 233–315. 1902.
245. WEBER, G. F. Nailhead spot of tomato caused by *Alternaria tomato* (Cke.) n. comb. Fla. Agr. Exp. Sta. Bul. 332. 1939.
246. WEI, C. T. Rust resistance in the garden bean. Phytopath. **27**: 1090–1105. 1937.
247. WEISS, F., C. R. ORTON, AND R. E. HARTMAN. Investigations of potato wart. U. S. Dept. Agr. Bul. 1156. 1923.
248. WELLMAN, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopath. **29**: 945–946. 1939.
249. WELLMAN, F. L., AND DOROTHY J. BLAISDELL. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bul. 705. 1940.
250. WHITE, R. P. Tomato wilt investigations. Kans. Agr. Exp. Sta. Tech. Bul. 20. 1926.
251. WILSON, J. J. The pathological relationship between the host and parasite in varieties and strains of watermelons resistant to *Fusarium niveum* E.F.S. Iowa Agr. Exp. Sta. Res. Bul. 195: 107–152. 1936.
252. WINGARD, S. A. The development of rust-resistant beans by hybridization. Va. Agr. Exp. Sta. Tech. Bul. 51. 1933.
253. ZAUMEYER, W. J. The bacterial blight of beans caused by *Bacterium phaseoli*. U. S. Dept. Agr. Tech. Bul. 186. 1930.
254. ———. Three previously undescribed mosaic diseases of pea. Jour. Agr. Res. **60**: 433–452. 1940.
255. ———, AND B. L. WADE. Varietal reaction of pea to pea-streak virus 1. Phytopath. **27**: 1009–1013. 1937.

THE BOTANICAL REVIE

VOL. VII OCTOBER, 1941 No. 10

APOMIXIS IN THE ANGIOSPERMS

G. L. STEBBINS, JR.
University of California

INTRODUCTION

Parthenogenesis, apospory and other substitutes for sexual reproduction have been regarded by most American botanists as "freak" phenomena. Their existence in a few species and genera is recognized, but the study of apomixis has too often been considered a side track which has little connection with the main lines of botanical research. Recent work has, however, shown that the study of apomixis by means of the modern method and principles of cytogenetic research will yield results which are both interesting in themselves and of great value in the solution of several major problems confronting botanists today.

The latest review of apomixis in plants, that of Rosenberg (1930), described all the important cases known at that time. The present reviewer aims partly to revise and enlarge Rosenberg's discussion, partly to show the connection between the problems of apomixis and other major problems.

TERMINOLOGY AND DESCRIPTION OF PHENOMENA

Several systems have been proposed for the classification of the phenomena of apomixis. That of Winkler (1908, 1920, 1934) has been most widely accepted, but is incomplete in the light of present knowledge. Stebbins and Jenkins (1939) and Fagerlind (1940*a*) have proposed revisions, of which the latter is the most complete. In the opinion of the present reviewer, Fagerlind's system is satisfactory in every way and should be generally adopted in order to secure uniformity in the description of these phenomena. It is represented in Figure 1 which is adapted from Table I of Fagerlind's paper. Of the several definitions of the term apomixis (Winkler, 1908; Edman, 1931; Darlington, 1937; Fagerlind, 1940*a*), the

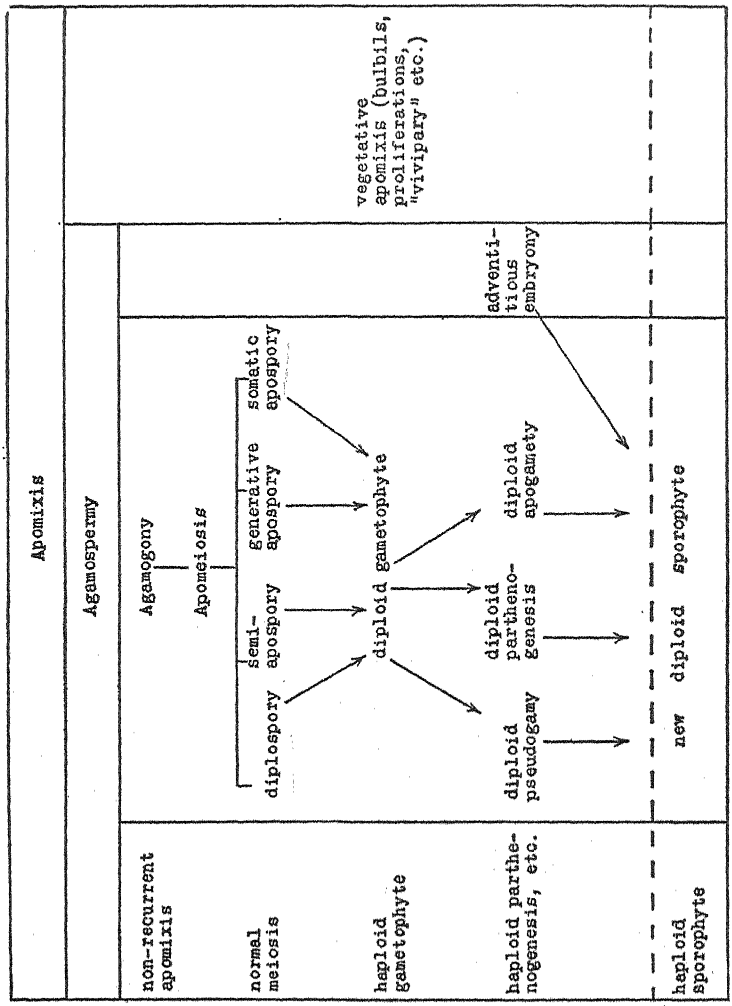


FIG. 1. Chart illustrating the interrelationships of the various apomictic processes.

original one of Winkler seems to the present writer the most factory. It may be translated as follows: "apomixis is the substitution for sexual reproduction of another, asexual reproductive process that does not involve nuclear or cellular fusion (that is, fertilization)." The two main types of apomixis are vegetative apomixis and agamospermy, or apomixis through seed production.

Naturally, not all types of vegetative reproduction can be termed apomixis, but only those that are substitutes for the sexual method. These include mainly the occurrence of vegetative buds, bulblets or proliferations in the place of flowers or inflorescences. Good reviews of the known cases are given by Ernst (1918) and Goebel (1931). Most botanists are familiar with such cases arious *Allium* spp., *Polygonum viviparum, Saxifraga stellaris* var. *comosa, Agave* spp., *Cyperus* spp. and the so-called "viviparous" races of various grasses (*cf.* Turesson, 1926, 1930, 1931; Flovik, 1938). The term vivipary should not be used for this type of proliferation, since it is properly applied to a very different phenomenon, namely, the germination of seeds while still upon the mother plant (Goebel, 1932). So far as the writer is aware, no careful developmental analysis of this type of reproduction has yet been attempted.

Agamospermy is in turn divided into three main categories: adventitious embryony, in which the new sporophyte is a direct proliferation from the ovular tissue of its parent (the term nucellar embryony is misleading, since the new embryo often develops from the inner ovular integument rather than from the nucellus); agamogony, in which alternation of generations occurs (although not in respect to chromosome number); and the non-recurrent types of apomixis, in which normal meiosis takes place and a haploid egg gives rise to a haploid sporophyte.

Adventitious embryony resembles vegetative reproduction in that the new sporophyte develops directly from the old, and the gametophyte, though usually formed, does not function. Many cases of this phenomenon were recorded in the earlier literature (Schnarf, 1929; Rosenberg, 1930), and more recently that of *Citrus* has been carefully studied (Swingle, 1932; Frost, 1926, 1938*a, b*) while several additional cases have been reported (see Table I). Archibald (1939) recognizes three types of adventitious embryo formation: 1) that dependent on fertilization and endosperm development, as in *Citrus* (Frost, 1926, 1938*a, b*); 2) that dependent on endo-

.n development but not on fertilization, as in *Coelebogyne* (*Alchornea*) *ilicifolia* (*cf.* Schnarf, 1929) ; 3) that dependent neither on fertilization nor on endosperm development, as in *Opuntia aurantiaca.* These types parallel the types of apomictic embryo formation from an egg, namely, pseudogamy and parthenogenesis. Webber (1940) has reviewed thoroughly the literature on adventitious embryony.

In *Ochna serrulata* (Chiarugi and Francini, 1930) diplospory and somatic apospory regularly take place, so that the gametophyte is normally diploid. Nevertheless, the egg cells most often fail to develop, and the parthenogenetic embryos, when formed, always degenerate. They are replaced by adventitious embryos formed from the inner integument of the ovule. These embryos begin growth some time after anthesis.

In spite of the fact that it eliminates practically all of the processes associated with sexual reproduction, adventitious embryony retains an important physiological characteristic of the sexual process, and of other types of agamospermy, namely, the ability to produce the distinctive characteristics of the seedling. In *Citrus* (Swingle, 1932; Frost, 1938*a;* Hodgson and Cameron, 1938) plants reproduced vegetatively by means of cuttings have the characteristics of the adult stock, while those grown from apomictically produced seed have all of the morphological features (cotyledons, thorns, *etc.*) as well as the vigor of growth found in seedlings of a normal sexual progeny. This shows that the appearance of juvenile characteristics is not the result of sexual reproduction, but is produced by the physiological environment within the developing ovule and young seed. Although Horn (1940) has suggested that the peculiar reduced nature of the embryo of *Garcinia mangostana* is due to its adventitious origin, Sprecher (1919) describes this type of embryo in several other species of the family Guttiferae, so that it appears to be characteristic of both sexual and apomictic members of the family.

The term agamogony was suggested by Fagerlind to cover all those types of apomixis in which the daughter sporophyte develops from a diploid gametophyte, so that a morphological alternation of generations is present. As was first pointed out by Winkler (1934) and has been emphasized by Stebbins and Jenkins (1939) and Fagerlind (1940*a*), any agamogonic cycle must involve two distinct

processes not found in the sexual cycle: first, a substitute for meiosis, and second, a substitute for fertilization. As is evident from Table I, there are several substitutes known for each process, and the two series are not interdependent. Thus an apomict, if parthenogenetic, must also be either aposporous, diplosporous or semiaposporous, but may be any one of these three. Similarly, an aposporous apomict, if fertile, must in addition be either parthenogenetic, pseudogamous or apogametous, but may be any one of the three. Failure to recognize this simple fact has led to great confusion in terminology in many discussions of apomixis.

The various substitutes for meiosis in apomicts which give a diploid gametophyte are collectively termed apomeiosis. Although many transitions between them exist, the four types recognized by Fagerlind are distinct enough to merit classification. Apospory is the development of a gametophyte by means of a series of purely mitotic divisions. In somatic apospory the embryo-sac initial is a purely somatic cell, usually of the chalazal region, but sometimes of the nucellus. This process has long been known in ferns (Steil, 1939) and in *Hieracium,* subgenus *Pilosella* (Rosenberg, 1930). The more recently reported cases of somatic apospory are in *Ochna serrulata* (Chiarugi and Francini, 1930), *Sorbus* spp. (Liljefors, 1934), *Malus hupehensis* (Dermen, 1936), *Leontodon hispidus* (Bergman, 1935*b*), *Crepis occidentalis, acuminata, etc.* (Stebbins and Jenkins, 1939; Stebbins and Babcock, 1939), *Hypericum perforatum* (Noack, 1939), and *Poa pratensis* (Akerberg, 1939; Tinney, 1940). In generative apospory the archesporial cell develops directly into the embryo-sac mother cell, so that its first division is a purely somatic one corresponding both in appearance and in the age of the ovule when it occurs, to the first division of the macrospore in the related sexual species (Stebbins, 1932*b;* Fagerlind, 1940*a*). The processes of meiosis and megaspore formation are therefore completely omitted. This is called by Rosenberg (1930) the *Antennaria* method of parthenogenesis. Conditions intermediate between somatic and generative apospory are found in species in which the distinction between archesporial and nucellar cells is not well defined, for example *Oxyria digyna* (Edman, 1929) and *Athraphaxis frutescens* (Edman, 1931).

Diplospory is the form of apomeiosis in which dyads are formed, and the semiheterotypic division (Rosenberg, 1927) takes place.

It is quite comparable to the formation of dyads of diploid microspores which sometimes takes place in the anthers of many hybrids and other plants with abnormal meiosis, and corresponds to the *Taraxacum* scheme of parthenogenesis (Rosenberg, 1930). Semi-apospory is an intermediate condition characterized by the presence of a pseudohomoeotypic division (Gustafsson, 1934*b*, 1935*a*), in which the chromosomes are strongly contracted as in meiosis, but are not at all paired. As Gustafsson (1935*a*) and Fagerlind (1940*a*) have both pointed out, there are various intermediate conditions between these four types of apomeiosis. In some species, such as *Wikstroemia indica* var. *viridiflora* (Fagerlind, 1940*b*), different types of apomeiosis are found in different ovules of the same plant.

In any cycle of recurrent apomixis, apomeiosis must be followed by one of three abnormal types of sporophyte development, namely, parthenogenesis, pseudogamy or apogamety. In some cases, such as *Artemisia nitida* (Chiarugi, 1926) and certain forms of *Oxyria digyna* (Edman, 1929), *Coreopsis bicolor* (Gelin, 1934) and *Leontodon hispidus* (Bergman, 1935*b*), none of these processes occurs, so that apomeiosis leads to non-functional embryo-sacs and sterile ovules. This shows that the two critical processes of the agamogonic cycle are not necessarily linked to each other. To distinguish them from the parallel processes which occur occasionally in normal haploid gametophytes (see below), these methods of sporophyte development from a diploid gametophyte are called unreduced parthenogenesis, pseudogamy, or apogamety.

Unreduced parthenogenesis, in which embryo development from the diploid egg is autonomous, is the most common method of sporophyte development in agamogonic plants. Although the course of embryo development is very similar to that of a sexual embryo, the time when the egg cell starts to divide varies in different genera. In many cases, such as *Hieracium, Erigeron, Ochna* (Chiarugi and Francini, 1930) and *Crepis* (Stebbins and Jenkins, 1939), the embryo has already begun to grow when the flowers first open, while in others, such as *Antennaria* (Stebbins, 1932*b*), it begins later, and in *Malus hupehensis* (Dermen, 1936) it is very much delayed. Endosperm development may precede or follow that of the embryo; both conditions have been described in the same species of *Chondrilla* (Poddubnaja-Arnoldi, 1933).

Unreduced pseudogamy as a regular stage in an apomictic cycle

is best known in *Potentilla* (Müntzing, 1928; Popoff, 1935; Gentscheff, 1938; Gentscheff and Gustafsson, 1940*b*), *Rubus* (Gustafsson, 1930; Crane, 1940) and *Poa* (Akerberg, 1936; 1939; Tinney, 1940; Engelbert, 1940). In all three of these genera the origin of the embryo sac is aposporic (*cf.* Gentscheff and Gustafsson, 1940*b*). These latter two authors have found that embryo development in *Potentilla* apomicts may begin autonomously, but that the endosperm develops only after fertilization. The "stimulus" provided by the pollen tube in this and probably other cases of pseudogamy is, therefore, the fertilization of the endosperm nucleus by one of the male nuclei. The same situation apparently exists in *Poa pratensis* (Tinney, 1940).

In a number of cases of facultative pseudogamy, the proportion of hybrid to apomictic offspring varies with the chromosome number of the pollen parent. Darrow and Waldo (1933) found that several tetraploid *Rubus* apomicts ($2n=28$) yielded few or no sexual offspring when selfed or crossed with other apomicts having the same chromosome number, but one of them, crossed with a diploid strain ($2n=14$), gave a majority of sexual hybrid offspring. Parallel results were obtained by Crane (1940), using the octoploid *Rubus vitifolius* ($2n=56$) as the ovulate, a diploid ($2n=14$) and a tetraploid ($2n=28$) form of *R. idaeus* as the pollen parent. Pollen from the diploid produced only pentaploid hybrids ($2n=35$), but that from the tetraploid produced both hybrids and purely matroclinous octoploid offspring. On the other hand, pollen from the hexaploid *R. loganobaccus* ($2n=42$) produced only hybrids with $2n=49$. Petrov (1939) found that unreduced eggs of a triploid form of *R. idaeus* ($2n=21$) produce hexaploid ($2n=42$) hybrids when crossed with *R. loganobaccus,* but that pollen from diploid *R. idaeus* produces exclusively triploid pseudogamous progeny. The results of Noack (1939) in *Hypericum* agree, in general, with those of Darrow and Waldo in *Rubus*. *H. perforatum,* a tetraploid ($2n=32$), forms only unreduced eggs by somatic apospory. Under self pollination these give rise almost exclusively to pseudogamous offspring, but when pollen from diploid ($2n=16$) species of *Hypericum* is used, pentaploid hybrids ($2n=40$) are often produced. The ability of a facultatively pseudogamous plant to produce apomictic or sexual offspring depends, therefore, both on the proportion of ovules in which viable reduced eggs are formed and on the

relationship between the chromosome number of the endosperm, egg and male gametes. For this latter relationship, however, no simple rule can be formulated which fits all the cases cited above. Apparently, other unknown factors are involved.

Apogamety (this term, coined by Renner, has been adopted by Fagerlind in preference to the more familiar apogamy, because of the indiscriminate use of the latter term in the older literature) is the development of an embryo from a nucleus of the gametophyte other than the egg. It occurs occasionally in few angiosperms, but no authentic case is known in this group of apogamety as a regular method of embryo formation. The cases of *Ochna serrulata* (Chiarugi and Francini, 1930) and *Athraphaxis frutescens* (Edman, 1931) are well established. The remarkable situation inferred by Jeffrey and Haertl (1939*a, b*) for *Trillium* needs further confirmation.

Reduced parthenogenesis, pseudogamy or apogamety results from the stimulation to development of the haploid egg cell of a gametophyte which has developed from a normal megaspore, the product of typical meïosis. Since these reduced apomictic processes usually produce sterile haploids, and are not repeated from generation to generation, they are termed non-recurrent apomixis. Darlington (1937) gives a complete list of the haploids then known in angiosperms, with the methods by which they were produced. A few (*Datura, Zea, Triticum*) were produced by means of cold or heat treatment, but the majority were obtained by the stimulation of pollen, either of a very different species, of its own pollen in a self-incompatible form, or of pollen treated with x-rays. Since in all of these latter cases the development of the egg was presumably stimulated by the action of some type of pollen incapable of fertilizing it, they should be considered cases of pseudogamy rather than parthenogenesis in the strict sense of the word. Redinger (1938) has produced completely homozygous diploids and tetraploids in *Petunia* by the application of pollen of *Salpiglossis*. As Redinger has demonstrated cytologically, these are cases of reduced pseudogamy with subsequent doubling of the chromosome number. The development of this method holds great possibilities for the plant breeder. If pseudogamous diploid progeny can be induced in large numbers in complex hybrids, true breeding homozygotes may be produced immediately, thus eliminating the long process of "fix-

ing" the valuable new combinations obtained from interracial or intervarietal hybridization.

Reduced pseudogamy often takes place at the same time as fertilization, so that a seed with two embryos results, giving rise to twin seedlings, one of which is haploid, the other diploid. Webber (1940) has already made a thorough review of this subject.

EVIDENCE FROM APOMIXIS AS TO THE NATURE OF MEIOSIS

The study of meiotic abnormalities found in apomicts is one of the most promising methods for an attack on the problem of the mechanism of meiosis in general, as has been pointed out by Gustafsson (1935*a*, 1938*a*, *b*, 1939*b*). In this connection the first fact to be emphasized is that the abnormalities of meiosis in the anthers of apomicts are not correlated with those found in their ovules (Gustafsson, 1938*a*). Darlington (1932) has attempted to show some points of parallelism between the male and female meiosis of apomicts, but his evidence is based on a few selected cases. His chief point is that apomicts with gametophyte formation by means of somatic apospory, and with pseudogamy or adventitious embryony, have, in general, fairly regular meoisis in the pollen mother cells, while diplospory or generative apospory in the ovules is usually accompanied by breakdown of meiosis in the anthers. In all groups, however, of which a large number of apomicts has been studied cytologically, there are now known to exist some apomicts with fairly normal and others with very abnormal or no microspore meiosis, regardless of whether the apomixis in these groups is facultative or obligate, or of the type of abnormalities which characterize the apomictic cycle. In *Hieracium* subg. *Pilosella,* which has facultatively somatic apospory followed by parthenogenesis, *H. aurantiacum* has fairly normal meiosis and pollen formation, while *H. excellens* has abnormal meiosis and no pollen (Rosenberg, 1917). In the American species of *Crepis,* with the same methods of reproduction, *C. occidentalis* (no, 2169) and *C. intermedia* have fairly normal meiosis, while in *C. acuminata* (no. 2163) the pollen mother cells degenerate before meiosis begins (Stebbins and Jenkins, 1939). In *Potentilla,* with facultative or obligate somatic apospory (probably) and pseudogamy, *P. argentea* has regular, *P. collina* and *P. hirta* irregular microspore meioses (Müntzing, 1928, 1931; Popoff, 1935). In *Taraxacum, Hieracium* subg. *Archieracium* and

Antennaria, all with diplospory or generative apospory followed by parthenogenesis, we have *T. Norstedtii, T. fulvum* and *T. melanthoides, H. umbellatum* and *A. fallax* with nearly normal P. M. C. meiosis, whereas several *Taraxaca, Hieracia* and *Antennaria canadensis* have very abnormal P. M. C. meiosis (*cf.* Gustafsson, 1938*a*). Furthermore, in the facultatively apomictic groups there is no evidence of correlation between the percentage of apomictic development and the amount of abnormality in the P. M. C.'s This is particularly clear in *Crepis* (Stebbins and Jenkins, 1939) in which the authors made a direct attempt to obtain such evidence. The meiotic abnormalities in the male and female cells must therefore be considered as separate phenomena.

Convincing evidence of this lack of correlation is presented by Gustafsson (1938*a*) who lists several species of *Taraxacum* and *Antennaria* in which the course of meiosis in the ovules is much more abnormal than in the anthers of the same plant. Since there is no doubt that the physiological disturbances which produce these abnormalities are quite different in the two sets of organs, the genetic factors controlling them are probably different. This point must be remembered in all future studies of apomixis; in the past, too much emphasis has been given by many workers to the meiotic abnormalities found in the pollen mother cells of apomicts.

As Gustafsson (1935*a*, 1938*a*, *b*; Gentscheff and Gustafsson, 1940*a*) has shown, the meiotic abnormalities in both the anthers and ovules of certain apomicts include a series of stages progressing from normal meiosis toward a typical somatic mitosis. In both cases the abnormalities are only in part due to the lack of homology between chromosomes. Rather, they are due to physiological alterations in the tissues concerned, the nature of which is not yet understood, but which should, when attacked from the experimental point of view, give us valuable clues as to what causes the differences between normal meiosis and a normal somatic mitosis.

Before the evidence now available can be applied to the current theories of meiosis, one point should be made clear. The P. M. C.'s and E. M. C.'s of certain apomicts, in particular *Hieracium* subg. *Archieracium,* differ not only in the degree of "mitotisation" (*i.e.,* conversion of meiosis into mitosis), but also in the way in which this mitotisation has occurred. In both sexes there are found, usually in different species or races, but sometimes in different flowers of

the plant, two types of division intermediate between meiosis and mitosis. These are the semi-heterotypic division (Rosenberg, 1927, 1930) and the pseudo-homeotypic division (Gustafsson, 1934*b*, 1935*a*). In the former, the chromosomes resemble in degree of contraction those of the first (heterotypic) division of meiosis, and, as in that division, do not pass normally to the poles, so that a restitution nucleus is formed. In the pseudo-homeotypic division the chromosomes are contracted as in meiosis, and are unpaired, but form a typical equatorial plate, split, and the daughter chromosomes pass normally to the poles, so that the division is in its effect an equational one, like an ordinary somatic mitosis. There are numerous transitions between these different types. Although Stebbins (1932*b*) considered that the first embryo-sac division in the generative apospory of *Antennaria* is a typical somatic mitosis, Gustafsson (1935*a*, 1939*b*) believes that it never is. This is because, even when the chromosomes at metaphase and anaphase are in shape and behavior typical of somatic mitosis, the nucleus at prophase and the premitotic resting stage is abnormally large and there is great "hydration" of the chromosomes. The writer has reexamined his slides in the light of Gustafsson's observations, but has not been able to verify them. In the sexual species of *Antennaria,* the mitotic prophase nucleus in the first division of the haploid megaspore is also very large; the prophase nucleus in the first embryo-sac division in the apomicts is 4–6 times larger, with 6 times as many chromosomes present. Furthermore, the "hydration" (weak staining, apparently small and diffuse granules) of the chromosomes in the resting nucleus is most evident in younger archesporial nuclei of the apomicts, and becomes progressively less as the onset of prophase approaches. Hence the writer reaffirms his opinion that the first division of the archesporial nucleus in typical generative apospory is a normal somatic mitosis. That this is true in the microsporocytes of some *Hieracium* apomicts is quite certain (Gustafsson, 1935*a*).

Two important facts are known which give a clue to the nature and causes of these physiological disturbances of meiosis. In the first place, they are usually, perhaps always, different in the ovules and anthers of the same species (Gustafsson, 1938*a*). Restitution nuclei and the semi-heterotypic division, although often found in the P. M. C. of *Hieracium* and *Antennaria,* have never been found

in the E. M. C. in these genera. Secondly, the abnormalities are always associated with a difference in the timing of the divisions with respect to the condition of the surrounding tissue, as well as in the relative growth and division rates of the tissues involved (Gentscheff and Gustafsson, 1940*a*). In *Hieracium* the somatic and semi-heterotypic types of division in the anthers take place when the sporocytes are relatively small and the tapetal cells have just begun their growth and division cycle; that is, at an early stage in anther and tapetal development compared to the time of normal meiosis. The "premeiotic" resting stage, as well as the prophase itself, are much curtailed, and the sporocytes fail to grow to normal size. The pseudo-homeotypic division in the microsporocytes of *H. amplexicaule* enters prophase at a developmental stage similar to that of normal meiosis, but the growth of both sporocytes and tapetal cells between early prophase and metaphase is considerably less in cells which undergo the pseudo-homeotypic division than in other sporocytes of the same apomict which form bivalents. Either the duration of prophase is abnormally short, or the growth rate of both sporocytes and tapetum during this stage is abnormally slow. A third type of abnormal division, found only in the microsporocytes of *H. robustum,* similar to the "double reproduction" of chromosomes well known in *Spinacia* and other genera, is associated with an abnormally great growth of the sporocytes, and a slow growth of the tapetum. In every case meiosis with bivalent formation can take place only when prophase is initiated at the same time, is of the same duration, and growth of both sporocytes and tapetum is the same as in the normal meiosis of the sexual species.

In the ovules of all apomicts with failure of meiosis, the megasporocyte divisions take place later than in their normal sexual relatives, and the greater the delay of the divisions, the more they resemble somatic mitosis (Gustafsson, 1935*a*, 1938*a*, *b*, 1939*b*; Gentscheff and Gustafsson, 1940*a*). The divisions, when they occur, are carried through very rapidly, and, as in the microsporocytes, the prophase is much shorter than in normal meiosis. The delay, a prolonged resting stage, is usually associated with considerable growth and hydration or vacuolisation of either the cytoplasm or the nucleus, or both. Gustafsson has, therefore, advanced the hypothesis that the delay in onset of meiosis has produced a conflict between the meiotic tendency and certain forces of vacuolisation which nor-

mally act on the developing megaspore. These forces are said to inhibit meiosis. Fagerlind (1940*b*) has criticized this hypothesis, mainly on the grounds that with our present lack of knowledge of the physiology of meiosis, the concept of vacuolisation forces has little meaning. In addition to this criticism, there can be cited some observations which indicate that the observed vacuolisation and hydration are incidental phenomena, rather than any direct indication of the cause of the failure of meiosis. Stebbins and Jenkins (1939) pointed out that whereas in generative apospory the hydration or vacuolisation affects first the nucleus, then the cytoplasm, in somatic apospory the reverse is the case, and the invading nucleus looks normal until it has already replaced the megasporocyte. If vacuolisation forces were the primary causal factors in both somatic and generative apospory, they should act similarly in the two cases.

The abnormal meiotic processes in apomicts provide direct evidence on the two principal hypotheses about the relation between mitosis and meiosis, namely, the precocity theory of Darlington (1932, 1937) and the retardation theory of Sax and Sax (1935). Darlington maintains that the chromosomes attract each other in pairs when single, but not when double; that normally the chromosome is single in the resting stage and divides before the onset of the mitotic prophase. The onset of meiotic prophase, however, is said to be precocious, so that the chromosomes have not yet divided and, being single, are able to pair. Sax and Sax, on the other hand, maintain that meiosis is occasioned by a retardation and prolongation of the prophase, permitting the chromosomes to uncoil more completely than they ever do in the meiotic prophase, so that linear point-by-point pairing is possible. Beasley (1938) has added the important observation that, while the onset of both mitotic and meiotic prophase is accompanied by a conspicuous growth and water intake of the nucleus, this process is much greater in the prolonged prophase of meiosis than in mitosis.

As was pointed out by Bergman (1935*a*, *b*), Darlington's hypothesis is well supported by behavior of the megaspore mother cells of many apomicts, which begin their first division abnormally late, and carry out either a semiheterotypic, a pseudohomeotypic, or a mitotic division. The situation in the anthers of *Hieracium*, however, is in direct contradiction to the precocity hypothesis, since there an abnormally early onset of prophase leads to the same mito-

sis-like abnormalities. In fact, no hypothesis which lays primary stress on the time of onset of the division can be reconciled with the situation in both the anthers and the ovules of apomicts.

On the other hand, the emphasis placed by Sax and Sax upon the condition of the entire nucleus during prophase is well supported by the evidence from apomicts. In every instance in which meiosis is replaced by some type of division in which pairing is omitted, whether the onset of the division is precocious or delayed, both the long duration of prophase and the sudden rapid growth of the nucleus are absent. In the anthers, both nuclei and cells usually remain abnormally small (Gentscheff and Gustafsson, 1940*a*). In the ovule the megaspore mother nuclei remain in the interphase condition at the time when meiosis would normally take place, and grow gradually. When they are ready to enter prophase, the normal time for meiosis is long past, and the initial nuclear size permits no such rapid growth as accompanies early meiotic prophase in sexual plants. This is clearly shown by observation of megaspore mother nuclei in *Antennaria* (Stebbins, 1932*b*) in which the ovules of neighboring florets may, on the one hand, go through an abnormal but in many ways characteristic type of meiosis, and, on the other, a prolonged resting stage followed by somatic mitosis and generative apospory. The nuclei which are in pachytene, prepared for meiosis, are many times larger than those in the resting stage and destined to undergo generative apospory.

To be sure, the phenomenon of meiosis cannot be really understood until the cause is discovered of the prophasic nuclear growth and chromosome uncoiling. Nevertheless, the suggestion of Oehlkers (1937), that meiosis and chromosome pairing are governed by the entire physiology of the plant, is a step in this direction which is well supported by the evidence from apomicts. In particular may be cited the relation between the behavior of the tapetal cells and the sporocytes in apomicts with abnormal meiosis.

Gentscheff (1937; Gentscheff and Gustafsson, 1940*a*) found that the abnormal divisions of the P. M. C.'s of some *Hieracium* apomicts were associated with an abnormally weak growth of the tapetal cells. Stebbins and Jenkins (1939) found that in an apomict of *Crepis acuminata* in which the P. M. C.'s always degenerate in early meiotic prophase, this degeneration is always preceded by the abnormal appearance and the degeneration of the tapetal cells.

Gustafsson (1939*b*) has, on the basis of these observations, advanced the hypothesis that the failure of meiosis is due to inhibiting substances released by the degenerating cells. An equally plausible alternative is that certain substances necessary for meiosis are normally transmitted to the P. M. C.'s by the tapetum, so that any influence, external or internal, which disturbs the normal function of these latter cells also inhibits meiosis. Two recent experiments make more likely the latter hypothesis. Zürn (1939) found that in flower stalks of *Oenothera* that were cut and placed in water, meiosis took place very quickly and chromosome pairing was reduced, while the abnormalities of both the tapetum and the tetrad nuclei resemble closely those found in *Hieracium* apomicts. On the other hand, stalks of the same plants placed in glucose solution were quite normal as to both meiosis and tapetal development. Gregory (1940) concluded from culture experiments with excised anthers and inflorescences that certain accessory substances necessary for meiosis are supplied by the vegetative organs of the plant. On this basis, the genetic complexes producing the abnormal meiosis of apomicts could act either through inhibiting the formation of these substances or through affecting the ability of the tapetal cells to absorb them and transmit them to the P. M. C.'s. At any rate, the problem of the relation between mitosis and meiosis is now open to experimental attack through the medium both of environmentally and of genetically controlled abnormalities.

APOMIXIS AND THE ALTERNATION OF GENERATIONS

As has been already stated by several students of the subject (Bower, 1935), our knowledge about apomixis has a definite bearing on the theories of the alternation of generations. In cases of stabilized recurrent apomixis, involving either apospory or apomeiosis, there is a regular alternation of morphological gametophyte and sporophyte generations without any change in the chromosome number. Furthermore, we now know many cases of haploid sporophytes and of diploid or polyploid gametophytes (*cf.* Wettstein, 1928), and in no case does the lowered or raised chromosome number make the sporophyte resemble in any way the gametophyte, or the gametophyte the sporophyte. In other words, the evidence from apomixis has demonstrated conclusively that the haploid-diploid chromosome cycle and the morphological alternation of

generations are not necessarily interdependent. Either one can take place without the other.

This concept is strengthened by the remarkable mutant form found by Andersson-Kottö (1932, 1936; Andersson-Kottö and Gairdner, 1936) in *Scolopendrium vulgare,* in which a single recessive gene produces a gametophyte-like aposporous plant. This worker has concluded that the morphological sporophyte and gametophyte are primarily physiologically controlled growth phases, which are normally integrated into the life cycle as a whole, and are usually associated with, but not caused by, the changes in the chromosome number.

Andersson-Kottö did not apply her findings to the theories of the alternation of generations, as recently discussed by morphologists (Bower, 1935; Eames, 1936), but this application may nevertheless be appropriately made here. Bower (1935) has rightly, in the opinion of the reviewer, pointed out that the terms "antithetic" and "homologous," as applied to the opposing theories for the nature of the alternation of generations, have in themselves caused much difficulty because they are not explicit, and has suggested that the terms "interpolation theory" and "transformation theory" be substituted. The use of these terms reduces the controversy to the definite historical question of how the alternating cycle originated, a question to which the answer lies outside the realm of cytogenetics. It is evident, however, from the facts stated above, that neither theory can rely for its support on cytological, genetical or physiological evidence. That in heterozygous "peculiar" plants of *Scolopendrium* the jump from sporophyte to spermatozoid can be taken with the gametophytic stage omitted shows that the interpolation of a gametophytic or a sporophytic stage into the life cycle by means of a genetic change or changes, is not inconceivable, while the presence in many apomicts of a morphological alternation of generations without alteration of the chromosome number shows that this could have been the original condition. Furthermore, the recent work on *Rubus* and *Hypericum* (Petrov, 1939; Noack, 1939) has shown that the egg of an aposporous diploid gametophyte can be fertilized and give rise to vigorous hybrid offspring. This shows that under certain conditions a gametophyte, both typical morphologically and normal in its physiological capabilities, can arise as a state in the vegetative somatic growth cycle of a sporophyte-like plant.

HYPOTHESES AS TO THE CAUSE OF APOMIXIS

To explain the appearance of apomixis in plants, four different agencies have been invoked. These are hybridization, polyploidy, genetic factors and necrohormones. The hybridization hypothesis of Ernst (1918) is now widely known, and is fully discussed elsewhere (Rosenberg, 1930; Darlington, 1932, 1937; Sharp, 1934; Gustafsson, 1935*a*). It is based on two assumptions: first, that all apomicts are of hybrid origin; and second, that the meiotic disturbances found in apomicts are occasioned by hybridity, and that these disturbances are responsible for the formation of diploid gametophytes and egg cells, or for the degeneration of haploid gametophytes. The first assumption holds nearly as well now as when first postulated. The great majority of apomictic forms among the higher plants are probably allopolyploids, or back-cross derivatives from allopolyploids, and therefore ultimately of hybrid origin (Stebbins, 1932*b;* Gustafsson, 1933; Babcock and Stebbins, 1938; Stebbins and Babcock, 1939). In *Crepis, Antennaria* and probably other genera (Babcock and Stebbins, 1938), many of the polyploid apomicts are morphologically very similar to certain diploid sexual species, so that the apomicts are, from the systematic point of view, intraspecific polyploids. Although this evidence points toward an autopolyploid origin for them, cytological studies of some of these same apomicts indicate an allopolyploid origin (Stebbins and Jenkins, 1939). In some cases, however, such as *Hieracium umbellatum* f. *apomictica* (Bergman, 1935*a*) and certain *Rubus* apomicts (Thomas, 1940), both the cytological and the taxonomic evidence suggests an autopolyploid or non-hybrid origin. Such apomicts may have been derived from sexual diploid forms by the direct doubling of the chromosome set.

The second assumption has become much more difficult to hold in the light of new discoveries about apomicts. As mentioned above, the meiotic abnormalities are often very different in the ovules from those found in the anthers. In a large number of apomicts of hybrid origin, the abnormalities which lead to the formation of diploid gametophytes are definitely greater than would be expected on the assumption that they are caused solely by lack of homology between the parental chromosomes (Gustafsson, 1935*a*), while in other cases these abnormalities are due wholly or in part to autopolyploidy (Thomas, 1940). They could not, therefore, be the re-

sult of hybridity alone. The view now held by most students of the subject (Rosenberg, 1930; Bergman, 1935*b;* Gustafsson, 1935*a;* Stebbins and Jenkins, 1939; Fagerlind, 1940*b*) is that hybridity is an accompanying phenomenon but not a causal agent of apomixis. The hybrid origin of most apomicts is not difficult to explain on other grounds. In hybrid derivatives, many of which are sterile as to their sexual reproduction, parthenogenesis is of high survival value, and so would be favored by natural selection (Darlington, 1937; Thomas, 1940). This is particularly important when we realize that many of these forms show hybrid vigor, or favorable new combinations of the characteristics of their parental species, which enable them to invade habitats not previously occupied by any of their relatives (*cf.* Babcock and Stebbins, 1938).

The close correlation between apomixis and polyploidy has been noted by all students of this subject. Darlington (1932, 1937) has listed four apomicts purportedly diploid, but his list must be modified since three of the species listed by him, *Chondrilla juncea* (Poddubnaja-Arnoldi, 1933), *Zephyranthes texana* (*cf.* Sato, 1938) and *Allium odorum* (Modilewski, 1930), are all now known to be polyploids. A revised list of apomictic or partially apomictic diploids includes the following species: *Alnus rugosa* (Woodworth, 1930), *Potentilla argentea formae* (Müntzing, 1928, 1931) and *P. arguta* (Popoff, 1935), various species of *Citrus, Poncirus* and *Fortunella* (Frost, 1925, 1926, 1938*b*) and *Eugenia* spp. (Pijl, 1934). The multitude of other apomicts now known are polyploids with anywhere from 3 to 15 times the basic haploid number for the genus; the last number in *Potentilla* (Clausen, Keck and Hiesey, 1940). The significance of this correlation is not yet understood. Polyploidy itself can not be the cause of apomixis, since the sexual polyploid plants greatly outnumber the apomicts. Furthermore, as Gustafsson (1935*a*) has pointed out, sexual polyploids exist in a number of genera, such as *Rubus, Rosa* and *Antennaria,* in which apomictic polyploids are also known. In *Rubus,* apomixis is prevalent in the European species of the subgenus *Eubatus,* but their close relatives in America, although mostly polyploid, are only rarely apomictic (Gustafsson, 1930; Crane and Thomas, 1939). In *Taraxacum* both autopolyploids (Kostoff and Tiber, 1939) and interspecific hybrids (Koroleva, 1939; Poddubnaja-Arnoldi, 1939) have been produced artificially from diploid sexual species of which

the natural polyploid relatives are all apomictic. None of these artificial creations showed any signs of apomixis. This is strong evidence that neither interspecific hybridization nor polyploidy alone is responsible for apomixis in this genus.[1]

The first evidence that apomixis may be at least in part governed by genetic factors was obtained by the experiments of Ostenfeld (1910) on *Hieracium*. That from the *Rubus* hybrids of Lidforss (1914), as interpreted by Gustafsson (1930) is, however, more definite. In *Rubus* there is evidently a single recessive factor which determines the presence or absence of pseudogamous development. On the other hand, Müntzing (1940) found that in a cross between a sexual and an apomictic biotype of *Poa alpina,* not only was the entire F_1 population apparently sexual, but, in addition, out of 31 F_2 plants tested, none was completely apomictic. Müntzing concludes, therefore, that the mechanism of inheritance of apomixis in *Poa* must be more complex than a single recessive gene. The presence in this genus of many biotypes showing all degrees of development of apomixis supports this conclusion.

Apospory without apomixis is conditioned by recessive genetic factors in *Scolopendrium* (Andersson-Kottö, 1932) and *Leontodon hispidus* (Bergman, 1935*b*). The aposporous *Leontodon* plant gave normal progeny after open pollination, but upon selfing segregated not only for apospory but for asynapsis as well, suggesting a genetic connection of some kind between the factors producing these two types of abnormality. The presence of recessive genes producing asynapsis and other meiotic abnormalities is now well known (*cf.* Sharp, 1934; Darlington, 1937). Since, as has been shown above, the presence of apomixis is correlated with and partly conditioned by abnormalities of meiosis produced by causes other than non-homology of the chromosomes, there is good reason to believe that the underlying tendencies toward apomixis are produced by genetic mechanisms similar in their effect on the plant to these known genes. That such genetic mechanisms may be widespread and not infrequent in the higher plants is suggested by the presence of occasional apospory in a number of normally sexual plants in addition to those already mentioned (*cf.* Bergman, 1935*b*). *Oxyria digyna* (Edman, 1929), *Antennaria dioica* (Haberlandt, 1923*b*), *Coreopsis bicolor* (Gelin, 1934) and *Picris hieracioides* (Bergman,

[1] The writer has not been able to see the paper of Christoff (1940) which apparently deals with this same subject.

1935*b*) are all examples of this. It is particularly significant, as Bergman has pointed out, that the majority of these examples are in the Compositae, a family which contains an exceptionally large number of apomictic genera.

The hypothesis now held by most students of apomixis, therefore, is that the original tendencies toward this process are genetically controlled, either by single recessive genes or more likely by constellations of complementary factors. Hybridization is probably important in bringing together complementary factors (*cf.* Rosenberg, 1930), and undoubtedly plays a large rôle through the production of vigorous sterile or partly sterile types in which apomixis has a very high selective value. Polyploidy is usually necessary for the full expression of these genes, and probably acts as a modifying agent by changing the physiological condition of the cells. This hypothesis is highly plausible and can be attacked experimentally from several different angles.

Haberlandt (1923*a*, *b*) developed the theory that the stimulus for apomixis is supplied by necrohormones produced by degenerating cells in the vicinity of those that develop. Gustafsson (1935*a*) and Stebbins and Jenkins (1939) have shown, however, that diploid gametophytes may start development by either diplospory or apospory before any of the somatic cells of the ovule has started to degenerate, indicating that the necrohormone theory cannot account for the apomeiotic development of the gametophyte. Gustafsson (1935*a*, 1938*a*) suggested that Haberlandt's hypothesis may have a limited application to the pseudogamous development of the diploid egg cell, but later evidence (Gentscheff and Gustafsson, 1940*b*) casts considerable doubt on even this suggestion.

APOMIXIS IN RELATION TO SPECIES DIFFERENTIATION AND PLANT DISTRIBUTION; THE AGAMIC COMPLEX

The development of apomixis in a group of plant species has the following two important effects on its evolution. First, by means of this process a species complex gives rise to innumerable microspecies, or "clonal species" (Darlington, 1932, 1937) which are genetically constant and are separated from their nearest relatives by the most minute differences. Second, through the perpetuation by apomixis of sexually sterile hybrid derivatives, the genetic and morphological barriers between the original species from which

the apomicts arose are obliterated, so that in any predominantly apomictic group distinct species comparable to those found in sexually reproducing organisms cease to exist (Dobzhansky, 1937; Babcock and Stebbins, 1938). These two facts have long been recognized as the underlying causes of the taxonomic difficulty of such genera as *Hieracium, Taraxacum, Antennaria,* and some sections of *Rubus*.

As a result of their studies on the largely apomictic American species of *Crepis*, Babcock and Stebbins (1938) concluded that the best way to analyze a species complex in which polyploidy and apomixis are prevalent is on the basis of its diploid sexual members. They found that among the apomictic forms an enormous number of "micro-species" could be recognized, all forming a more or less continuous series of intergrading forms, but that not a single one of these possessed any new morphological characteristics. Every feature of the apomicts could be explained either as a result of the recombination of characteristics found in two or more of the seven sexual diploids, or as the direct result of an increase in the chromosome number. Furthermore, the seven diploids are very distinct from each other. The difficulties which botanists have had in distinguishing between these species are due entirely to the presence of auto- and allopolyploid apomicts. Such a species complex was called an *agamic complex* (Babcock and Stebbins, 1938). Agamic complexes are probably numerous and widespread throughout the angiosperms. The following is a list of genera in which the presence of an agamic complex is definitely established (listed in capital letters) or may be inferred (listed in italics). There are, of course, species groups in all of these genera in which apomixis does not occur. For the method of apomixis, the following abbreviations are used: V = vegetative apomixis; AE = adventive embryony; SA = somatic apospory; GA = generative apospory; D = diplospory or semiapospory; P = parthenogenesis; Ps = pseudogamy. In some genera more than one method occurs. The reference list aims to be complete only for the more recent publications; the older references are purposely omitted when they are well reviewed by Ernst (1918), Schnarf (1929) and Rosenberg (1930).

Several other genera are listed by Schnarf which may contain regularly apomictic species, but the evidence in these is either disproved or needs further confirmation. Among these are *Notho-*

TABLE I

AGAMIC COMPLEXES IN ANGIOSPERMS

FAMILY	GENUS	METHOD	REFERENCES
Gramineae	FESTUCA	V	Turesson, 1926, 1930, 1931; Flovik, 1938
	POA	V; SA, GA + Ps	Müntzing, 1933, 1940; Akerberg, 1936, 1939; Kiellander, 1935, 1937; Flovik, 1938; Brown, 1939; Tinney, 1940; Tinney and Aamodt, 1940; Engelbert, 1940
	Deschampsia	V	Flovik, 1938
	Calamagrostis	GA + P	Stenar, 1935
Liliaceae	*Allium*	V; AE + P	Haberlandt, 1923*a*, *b*; Modilewski, 1930; Cappeletti, 1931; Weber, 1929; Levan, 1940
	Funkia (*Hosta*)	AE	Schnarf, 1929; Rosenberg, 1930
	Zephyranthes	GA + Ps	Pace, 1913; Sato, 1938
Burmanniaceae	*Thismia*	GA + P	Ernst, 1918; Schnarf, 1929; Rosenberg, 1930
Orchidaceae	*Nigritella*	AE	Afzelius, 1928, 1932
	Zygopetalum	AE	Suessenguth, 1923
Saururaceae	*Houttuynia*	D, GA + P	Okabe, 1930
Betulaceae	*Alnus*	AE	Woodworth, 1930
Urticaceae	*Elatostema*	GA + P	Ernst, 1918; Schnarf, 1929; Rosenberg, 1930
Balanophoraceae	*Balanophora*	GA + P	Ernst, 1918; Schnarf, 1929; Rosenberg, 1930; Zweifel, 1939
Polygonaceae	*Athraphaxis*	AE; SA + P	Edman, 1931
Ranunculaceae	*Ranunculus*	?	Marsden-Jones and Turrill, 1935; Metcalfe, 1939
Rosaceae	*Pyrus*, subg. *Malus*	SA + P	Dermen, 1936
	Pyrus, subg. *Sorbus*	SA + P	Liljefors, 1934
	POTENTILLA	SA + Ps	Müntzing, 1928, 1931; Popoff, 1935; Gentscheff, 1938; Gentscheff and Gustafsson, 1940*b*; Clausen, Keck and Hiesey, 1940

TABLE I (*Continued*)

FAMILY	GENUS	METHOD	REFERENCES
	RUBUS	SA + Ps	Focke, 1910–14; Lidforss, 1914; Gustafsson, 1930, 1939*a;* Darrow and Waldo, 1933; Vaaramo, 1939; Petrov, 1939; Crane and Thomas, 1939; Thomas, 1940
	Alchemilla	AE, SA + Ps	Rosenberg, 1930; Gustafsson, 1935*a*, 1939*b;* Gentscheff and Gustafsson, 1940*b*
	Rosa, sect. *canina*	AE?	Täckholm, 1922; Schnarf, 1929; Gustafsson, 1931*a, b*
Rutaceae	CITRUS	AE	Frost, 1925, 1926, 1938*a, b;* Swingle, 1932; H. J. Webber, 1931, 1932; J. M. Webber, 1940
Euphorbiaceae	*Coelebogyne (Alchornea)*	AE	Schnarf, 1929; Rosenberg, 1930
	Euphorbia	AE	Schnarf, 1929
Buxaceae	*Sarcococca*	AE	Schnarf, 1929; Wiger, 1930
Anacardiaceae	*Mangifera*	AE	H. J. Webber, 1931; Juliano and Cuevas, 1932; Juliano, 1934, 1937
Ochnaceae	*Ochna*	AE	Chiarugi and Francini, 1930
Guttiferae	*Garcinia*	AE	Sprecher, 1919; Horn, 1940
Hypericaceae	*Hypericum*	SA + Ps	Noack, 1939
Cactaceae	*Opuntia*	AE	Schnarf, 1929; Archibald, 1939
Myrtaceae	*Eugenia*	AE	Schnarf, 1929; Pijl, 1934; Johnson, 1936
Thymeleaceae	WIKSTROEMIA	D, GA + P	Fagerlind, 1940*b*
Gentianaceae	*Cotylanthera*	D + P	Oehler, 1927 (?*cf.* Gustafsson, 1935*a*)
Compositae	*Eupatorium*	GA + P	Holmgren, 1919
	ERIGERON	D, GA + P	Holmgren, 1919; McDonald, 1927; Schnarf, 1929; Rosenberg, 1930; Oka, 1935

TABLE I (*Continued*)

Family	Genus	Method	References
	ANTENNARIA	GA + P	Rosenberg, 1930; Stebbins, 1932*a*, *b*, 1935; Bergman, 1935*c*, 1937
	Arnica	GA + P	Afzelius, 1936
	CHONDRILLA	D + P	Poddubnaja-Arnoldi, 1933
	TARAXACUM	D + P	Schnarf, 1929; Rosenberg, 1930; Gustafsson, 1932, 1935*a*, *b*, 1937; Poddubnaja-Arnoldi and Dianova, 1934. 1937; Turrill, 1938*a*, *b*; Koroleva, 1939; Erlandsson, 1939
	CREPIS (American spp.)	SA + P	Hollingshead and Babcock, 1930; Babcock and Stebbins, 1938; Stebbins and Jenkins, 1939; Stebbins and Babcock, 1939
	Youngia, sect. *Crepidopsis*		Hollingshead and Babcock, 1930; Babcock and Cameron, 1934; Babcock and Stebbins, 1937
	Ixeris	GA + P	Okabe, 1932
	HIERACIUM	SA, GA + P	Ostenfeld, 1910, 1921; Rosenberg, 1917, 1927, 1930; A. H. Gustafson, 1933; Christoff and Popoff, 1933; A. Gustafsson, 1934*a*, 1935*a*, *b*; Gentscheff, 1937; Gentscheff and Gustafsson, 1940*a*

scordum, Spiranthes, Ficus, Helosis (*cf.* Fagerlind, 1928), *Calycanthus, Thalictrum* (*cf.* Kuhn, 1928) and *Erophila.*

The systematic structure of an agamic complex can be understood only when its diploid sexual members are all known, and the limits of their variability have been determined. These may be looked upon as the pillars of the complex, while the apomicts represent its vast, intricate superstructure. Only in the relatively small and geographically restricted complex of *Crepis* has this type of analysis been carried out, although in *Rubus, Antennaria* and *Taraxacum* considerable progress has been made in this direction.

The primary causes of the polymorphism of agamic complexes are, therefore, hybridization, polyploidy and apomixis. Their origin may be pictured in the following manner. In the first place is a group of diploid species, each one well defined systematically, quite distinct from all the others and forming more or less sterile hybrids with them. The species then intercross, and either by somatic or gametic doubling, allopolyploids are produced. An alternative is the initial formation of autopolyploids and subsequent hybridization; either order of events leads to the same eventual result. Then, through the action of complexes of complementary genes brought together by hybridization, by the selection of successive mutations, or by some similar process, apomixis develops. There has been some discussion as to whether the onset of apomixis in such complexes is gradual or sudden. The gradual evolution of this process seems most likely in those complexes in which somatic apospory is present, such as *Hieracium* subg. *Pilosella, Potentilla, Crepis* and *Poa*. These complexes contain many facultative apomicts which regularly produce some sexual offspring. In *Crepis* there is evidence that the obligate apomicts have arisen from the facultative ones (Babcock and Stebbins, 1938).

In those with diplospory or generative apospory obligate apomicts are much more frequent, or are the only types known, as in *Hieracium* subg. *Archieracium* and *Taraxacum* (Gustafsson, 1935*a*). Gustafsson (1935*a*) follows Ernst (1918) in the belief that apomixis has arisen all at once in these groups. His chief argument in favor of this is that "an entirely new type of division had to be introduced in the peculiar kind of parthenogenesis occurring in *Antennaria* and *Hieracium*." In the opinion of the reviewer this argument is not very strong in the light of Gustafsson's own observations. The "mitotised" divisions which produce the diploid gametophytes in the ovules of these apomicts are merely extreme forms of a series grading toward normal meiosis. Furthermore, the nature of the division is dependent partly upon both the external and the internal environment. Hence, although no such form has yet been found, the existence of a facultative apomict in which some ovules produce diploid gametophytes by means of diplospory, and others haploid ones by means of meiosis, can be postulated without difficulty. Gustafsson's second argument, that in these groups there occur only apomicts derived directly from

sexual forms, and with triploid or tetraploid chromosome numbers, does not hold for *Antennaria* or for recent observations on *Taraxacum* (Erlandsson, 1939). Babcock and Stebbins (1938) have suggested that in most or all agamic complexes facultative apomicts precede obligate ones in the development of the complex. The presence of exclusively obligate apomicts in a complex or a section of one may indicate that the group is relatively old, and considerably removed from its sexual ancestors. In the parthenogenetic *Antennarias,* staminate plants, which could arise most easily by occasional sexual reproduction, are usually most frequent within or near the ranges of the sexual ancestors of these apomicts, suggesting that facultative apomicts exist only or chiefly in these regions. The question of the gradual or sudden onset of apomixis is one of the many which must be answered by future observation and experimentation.

The importance of this question lies in its bearing on the origin of polymorphism in agamic complexes. In complexes with initial facultative apomixis, occasional back- and out-crossing, with subsequent segregation, best explain this polymorphy, and this explanation fits very well the pattern of variation found in *Crepis* (Babcock and Stebbins, 1938). If, however, obligate apomixis sets in immediately after chromosome doubling, either in a species or an F_1 hybrid, the secondary back-cross and triple or quadruple hybrid types which appear to be characteristic of agamic complexes can not be formed. Polymorphism could arise, however, in three different ways. First, there might exist fertile sexual allopolyploids, of which various biotypes could from time to time become apomictic. Such sexual forms are now known to exist in *Poa* (Müntzing, 1940) and *Antennaria* (Gustafsson, unpubl.). Second, there might be occasional loss or non-disjunction of chromosomes in one of the critical divisions of the apomictic cycle, leading to variant aneuploids. This process may have been very important in *Poa alpina* and *Poa pratensis,* in which many aneuploid clones exist (Müntzing, 1933, 1940). Third, the process of "apogamic mutation," found by Ostenfeld (1921) in an obligate apomict of the subgenus *Archieracium,* may take place. Darlington (1932, 1937) has sought to explain these "mutations" as the result of pairing and chiasma formation in dissimilar chromosomes, with subsequent formation of restitution nuclei and the segregation in the following

division of the new crossover chromosomes. This explanation has been accepted by Bergman (1935*a, b*). Gustafsson, however, although he examined "tens of thousands of ovules" (1935*a*) of *Hieracium* at the correct stage, found only 19 prophases with the chromosomes differentiated, and says "there is no evidence of any chromosome pairing at prophase." This reviewer has examined thousands of ovules of *Antennaria* without finding restitution nuclei (Stebbins, 1932*b*). In this genus the division of the archesporial cell is essentially mitotic, or there is an abnormal meiosis which always leads to tetrad formation (Bergman, 1935*c*). There is, therefore, no evidence that the processes postulated by Darlington take place in the formation of the apomictic female gametophyte of these genera.

In *Citrus* (Frost, 1926) there are cases of undoubted apomictic mutation which can not be explained on Darlington's hypothesis, since the apomictic embryos are in this genus adventitious. The most frequent mutants found were albino or variegated seedlings, but some mutations from navel and seedless to non-navel and seedy fruits were also found. These were interpreted as due first to the highly heterozygous condition of the clones, which would make possible the manifestation of many recessive genes simply by the mutation of their dominant alleles; and second, to the possible chimaeric condition of the tissue from which the mutant embryos arose, which would make possible the occurrence of a genetic change at almost any stage in the development of this tissue. The latter hypothesis is strongly supported by the occasional appearance of tetraploid adventitious embryos. Frost has concluded (1938*a*) that "nucellar embryony, by favouring the evolution of excessive heterozygosis, has been a dominant agency in establishing the remarkable generative and somatic variation of *Citrus*." This conclusion may be of wide application to agamic complexes in general.

The systematic treatment of the agamic complex is one of the most difficult problems confronting the taxonomist. The method which has been widely adopted by Scandinavian botanists, who have done most of the work on these complexes, has been to describe each apomict as a distinct species. This practice has been defended by Du Rietz (1930) and more recently by Marklund (1938) and Darlington (1940) on the following grounds. First, different sexual species, even within the same genus, may differ

widely from each other in the amount of intraspecific variation that they contain, so that there is nothing inconsistent in the recognition of absolutely constant species separated from each other by minute differences, provided there is no intergradation. Second, if the apomicts are not regarded as distinct species, there are no larger entities which can be clearly separated from each other. The definition of species as entities separated from each other by a series of morphological characteristics which show no intergradation or overlapping is impossible in agamic complexes unless the individual apomicts are considered as species. The explanation for this fact is, of course, the hybrid origin of a majority of the apomicts.

Although these arguments themselves have not been refuted, the practice of recognizing individual apomicts as species of the same taxonomic rank as the species of sexual groups has been attacked by Müntzing, Tedin and Turesson (1931), Fernald (1933), Turrill (1938*a*, *b*) and Babcock and Stebbins (1938). The first objection which has been raised is that the recognition of apomicts as species has produced such a bewildering swarm of "species" that none but the specialist can have the remotest conception of their interrelationships. There is good reason to believe that even the *Taraxacum* and *Hieracium* specialists, or at least a considerable number of them, have focused their attention so long on minute differences that they can not fully understand their genera as wholes; "they can not see the forest for the trees." Secondly, in complexes containing sexual and apomictic forms which parallel each other in their morphological variation, the two standards of species delimitation become absurdly incongruous. In *Crepis acuminata*, for instance (Babcock and Stebbins, 1938), and in *Poa alpina* (Müntzing, 1940) there is a series of autopolyploid apomicts of which the morphological variation is entirely within the range of the sexual form. The recognition of these apomicts as species would create a series of species which would be morphologically indistinguishable from another series of completely interfertile biotypes. A similar situation was found by Gustafsson (1937) in a sexual population of *Taraxacum*, and very probably exists in other species containing autopolyploid apomicts, such as *Hieracium umbellatum*.

Babcock and Stebbins (1938) have proposed another method of treating these complexes, in which the different sexual forms are the basis of species delimitation. This reduces the number of

species enormously, and makes the complexes more nearly comparable systematically to the related sexual groups. The apomicts may be described if they are important for any reason, particularly in connection with problems of plant geography (see below), but are not given taxonomic recognition, so that they will not clutter up the indices and floras. One drawback to this method is that an intimate knowledge of the entire complex is necessary before the method can be applied to any part of it. The sexual forms may be very restricted in distribution, and may occur in such remote regions that they are not available for study, but until they are all known, the complex can not be understood. If, as is quite possible, some of the sexual ancestors are extinct, the complex becomes particularly difficult to analyze, and the method may have to be modified.

Agamic complexes, although they most certainly are anomalous or "freakish" from the systematic point of view, nevertheless deserve careful study, since they can provide us with much valuable information on plant distribution, both present and past (Gustafsson, 1935*b*; Turrill, 1938*a, b*; Babcock and Stebbins, 1938). In the first place, a large part of their phylogenetic history can be placed on a sound factual rather than a speculative basis. There is no doubt that the apomicts have been derived from the sexual members of the complex; hence the geographic regions or climatic provinces occupied by the sexual species are those from which the complex originated, while those inhabited by apomicts only have been occupied relatively recently. Thus northeastern California has been established as the main center of origin of the *Crepis* complex, while its members in the Rocky Mountains are all derived. The centers of origin of some other agamic complexes is also suggested by what we know of the distribution of their sexual members. The central Appalachian mountain region is the center for *Antennaria* in temperate eastern America, and contains five sexual species (Stebbins, 1935), while additional centers for this large agamic complex are in the central Rocky Mountains, the Sierra Nevada and Alaska. Most of the *Taraxacum* species known to be sexual center about southeastern Europe and southwestern Asia (*cf.* Gustafsson, 1932), which is undoubtedly the center of origin for the genus, but the occurrence of three sexual species in the Thian-Shan mountains of west central Asia (Poddubnaja-Arnoldi and Dianova, 1934) suggests that this may be an important secondary center, and

perhaps one in which many of the widespread groups of Arctic apomicts originated.

Furthermore, apomicts are excellent material for experiments on the effect of the environment on the genotype (Curtis, 1940) and on natural selection (Sukatschev, 1932). Also, because of their narrow range of tolerance and their morphological uniformity, they serve as excellent indicators of plant communities and as key species for the tracing of plant migrations (Gustafsson, 1935*b;* Babcock and Stebbins, 1938; Stebbins and Babcock, 1939). Also, on the basis of their populations of apomicts, the relative ages of different floras can be estimated. Those which are young should have a large proportion of apomicts, while the older floristic regions should contain the sexual members of several different agamic complexes. In this connection it is interesting to note that the distributional centers of the American *Crepis* complex in northeastern California and of the eastern American *Antennaria* in the Appalachians are both areas considered by plant geographers to have an ancient flora (Babcock and Stebbins, 1938; Fernald, 1931). On the other hand, 5 of the 12 genera listed by Fernald as "heteromorphic tangles" found in the youthful areas of the eastern United States are known to include agamic complexes, while the flora of the recently glaciated Scandinavian region has also a very large proportion of apomicts. The application of the distribution of apomicts to several specific problems in the plant geography of the western United States has been made by Babcock and Stebbins (1938) while Gustafsson (1935*b,* 1939*a*) has made similar use of the apomicts of *Taraxacum* and *Rubus* in Scandinavia.

From the evolutionary point of view, the agamic complex is a "closed system" which will never give rise to anything except new combinations of a given series of characteristics. Apomicts are splendid "opportunists"; due to the remarkable polymorphism of the complexes and often the hybrid vigor of the apomicts, they are frequently aggressive weeds, like the dandelions and hawkweeds. They are, however, adapted to taking great advantage of conditions prevailing when they come into existence, rather than to adjusting themselves to changing conditions over a long period of time. When separated from their sexual ancestors, apomicts will in time become relic species and die out (Babcock and Stebbins, 1938). Apomixis, therefore, is not a major factor in evolution, however

important it is in increasing the polymorphism and the geographic distribution of the genera in which it is found.

LITERATURE CITED

AFZELIUS, K. 1928. Die Embryobildung bei *Nigritella nigra*. Svensk Bot. Tidskr. **22**: 82–91.

———. 1932. Zur Kenntnis der Fortpflanzungsverhältnisse und Chromosomenzahlen bei *Nigritella nigra*. Svensk Bot. Tidskr. **26**: 365–369.

———. 1936. Apomixis in der Gattung *Arnica*. Svensk Bot. Tidskr. **30**: 572–579.

AKERBERG, E. 1936. Studien über die Samenbildung bei *Poa pratensis* L. Bot. Not. (Lund) **1936**: 213–280.

———. 1939. Apomictic and sexual seed formation in *Poa pratensis*. Hereditas **25**: 359–370.

ANDERSSON-KOTTÖ, I. 1932. Observations on the inheritance of apospory and the alternation of generations. Svensk Bot. Tidskr. **26**: 99–106.

———. 1936. On the comparative development of alternating generations, with special reference to ferns. Svensk Bot. Tidskr. **30**: 57–78.

———, AND GAIRDNER, A. E. 1936. The inheritance of apospory in *Scolopendrium vulgare*. Jour. Genet. **32**: 189–228.

ARCHIBALD, E. E. A. 1939. The development of the ovule and seed of jointed cactus (*Opuntia aurantiaca* Lindley). South Afr. Jour. Sci. **36**: 195–211.

BABCOCK, E. B., AND CAMERON, D. R. 1934. Chromosomes and phylogeny in *Crepis*. II. The relationships of one hundred eight species. Univ. Calif. Publ. Agr. Sci. **6**: 287–324.

———, AND STEBBINS, G. L., JR. 1937. The genus *Youngia*. Carnegie Inst. Wash. Publ. 484.

———, AND ———. 1938. The American species of *Crepis*: their relationships and distribution as affected by polyploidy and apomixis. Carnegie Inst. Wash. Publ. 504.

BEASLEY, J. O. 1938. Nuclear size in relation to meiosis. Bot. Gaz. **99**: 865–871.

BERGMAN, B. 1935*a*. Zytologische Studien über sexuelles und asexuelles *Hieracium umbellatum*. Hereditas **20**: 47–64.

———. 1935*b*. Zytologische Studien über die Fortpflanzung bei den Gattungen *Leontodon* und *Picris*. Svensk Bot. Tidskr. **29**: 155–301.

———. 1935*c*. Zur Kenntnis der Zytologie der Skandinavischen *Antennaria*-Arten. Hereditas **20**: 214–226.

———. 1937. Eine neue apomiktische *Antennaria*. Svensk Bot. Tidskr. **31**: 391–393.

BOWER, F. O. 1935. Primitive land plants.

BROWN, W. L. 1939. Chromosome complements of five species of *Poa*, with an analysis of variation in *Poa pratensis*. Am. Jour. Bot. **26**: 717–723.

CAPPELLETTI, C. 1931. Sull' azione dei prodotti del ricambio di miceli microrizogeni sulle piante ospiti. Ann. Bot. Roma **19**: 1–62.

CHIARUGI, A. 1926. Aposporia e apogamia in *Artemisia nitida* Bertol. Nuov. Giorn. Bot. Ital. n.s. **33**: 501–626.

———, AND FRANCINI, E. 1930. Apomissia in *Ochna serrulata* Walp. Nuov. Giorn. Bot. Ital. n.s. **37**: 1–250.

CHRISTOFF, M. 1940. Über die Fortpflanzungsverhältnisse bei einigen Arten der Gattung Hieracium nach einer experimentell induzierten Chromosomenvermehrung. Planta, Arch. Wiss. Bot. **31**: 73–90.

CHRISTOFF, M., AND POPOFF, A. 1933. Cytologische Studien über die Gattung *Hieracium*. Planta **20**: 440–447.

CLAUSEN, J., KECK, D. D., AND HIESEY, W. M. Experimental studies on the nature of species. I. The effect of varied environments on western North American plants. Carnegie Inst. Wash. Publ. 520.
CRANE, M. B. 1940. Reproductive versatility in *Rubus*. I. Jour. Genet. **40**: 109–118.
———, AND THOMAS, P. T. 1939. Segregation in asexual (apomictic) offspring in *Rubus*. Nature **143**: 684.
CURTIS, W. M. 1940. The structure and development of some apomicts of *Taraxacum*. Bull. Misc. Inf. Kew **1940**: 1–29.
DARLINGTON, C. D. 1932. Recent advances in cytology.
———. 1937. Recent advances in cytology.
———. 1940. Taxonomic species and genetic systems. *In* The new systematics: 137–160.
DARROW, G. M., AND WALDO, G. F. 1933. Pseudogamy in blackberry crosses. Jour. Hered. **24**: 313–315.
DERMEN, H. 1936. Aposporic parthenogenesis in a triploid apple, *Malus hupehensis*. Jour. Arn. Arb. **17**: 90–105.
DOBZHANSKY, TH. 1937. Genetics and the origin of species.
DU RIETZ, G. E. 1930. The fundamental units of biological taxonomy. Svensk Bot. Tidskr. **24**: 333–426.
EAMES, A. J. 1936. Morphology of vascular plants, lower groups.
EDMAN, G. 1929. Zur Entwicklungsgeschichte der Gattung *Oxyria* Hill, nebst zytologischen, embryologischen, und systematischen Bemerkungen über einige andere Polygonaceen. Acta Horti Bergiani **9**: 165–291.
———. 1931. Apomeiosis und Apomixis bei *Athraphaxis frutescens* C. Koch. Acta Horti Bergiana **11**: 13–66.
ENGELBERT, V. 1940. Reproduction in some *Poa* species. Canad. Jour. Res. Ser. C **18**: 518–521.
ERLANDSSON, S. 1939. The chromosome numbers of some *Taraxacum* species. Bot. Not. (Lund). **1939**: 261–264.
ERNST, A. 1918. Bastardierung als Ursache der Apogamie im Pflanzenreich.
FAGERLIND, F. 1938. Bau und Entwicklung der floralen Organe von *Helosis Cayennensis.*. Svensk Bot. Tidskr. **32**: 139–159.
———. 1940*a*. Die Terminologie der Apomixis-Prozesse. Hereditas **26**: 1–22.
———. 1940*b*. Zytologie und Gametophytenbildung in der Gattung *Wikstroemia*. Hereditas **26**: 23–50.
FERNALD, M. L. 1931. Specific segregations and identities in some floras of eastern North America and the Old World. Rhodora **33**: 25–63.
———. 1933. Recent discoveries in the Newfoundland flora. Rhodora **35**: 369–386.
FLOVIK, K. 1938. Cytological studies of arctic grasses. Hereditas **24**: 265–276.
FOCKE, W. O. 1910–1914. Species Ruborum. Monographiae generis Rubi prodromus. Bibl. Bot. **17**(72): 1–223; **19**(83): 1–274.
FROST, H. B. 1925. The chromosomes of *Citrus*. Jour. Wash. Acad. Sci. **15**: 1–3.
———. 1926. Polyembryony, heterozygosis, and chimeras in *Citrus*. Hilgardia **1**: 365–402.
———. 1938*a*. Nucellar embryony and juvenile characters in clonal varieties of *Citrus*. Jour. Hered. **29**: 423–432.
———. 1938*b*.. The genetics of *Citrus*. Cur. Sci., special number on "Genetics": 24–27.
GELIN, O. E. V. 1934. Embryologische und cytologische Studien in Heliantheae-Coreopsidinae. Acta Horti Bergiani **11**: 99–128.

——. 1938. Über die pseudogame Fortpflanzung bei *Potentilla.* Genetica **20**: 398–408.

——, AND GUSTAFSSON, Å. 1940*a*. The balance system of meiosis in *Hieracium.* Hereditas **26**: 209–249.

——, AND ——. 1940*b*. Parthenogenesis and pseudogamy in *Potentilla.* Bot. Not. (Lund) **1940**: 109–132.

GOEBEL, K. 1931. Blütenbildung und Sprossgestaltung. Organographie der Pflanzen, Suppl. 2.

——. 1932. Organographie der Pflanzen **3**(1): 1405–1408.

GREGORY, W. C. 1940. Experimental studies on the cultivation of excised anthers in nutrient solution. Am. Jour. Bot. **27**: 687–692.

GUSTAFSON, ALTON H. 1933. Cytological studies in the genus *Hieracium,* Bot. Gaz. **94**: 512–533.

GUSTAFSSON, ÅKE. 1930. Castrierung und Pseudogamie in der Gattung *Rubus.* Bot. Not. (Lund) **1930**: 477–494.

——. 1931*a*. Sind die *canina*-Rosen apomiktisch? Bot. Not. (Lund) **1931**: 21–30.

——. 1931*b*. Weitere Kastrierungsversuche in der Gattung *Rosa.* Bot. Not. (Lund) **1931**: 350–354.

——. 1932. Zytologische und Experimentelle Studien in der Gattung *Taraxacum.* Hereditas **16**: 41–62.

——. 1934*a*. Die Entwicklungswege der parthenogenetischen E. M. Z. Bot. Not. (Lund) **1934**: 333–338.

——. 1934*b*. Die Formenbildung der total Apomikten. Hereditas **19**: 259–283.

——. 1935*a*. Studies on the mechanism of parthenogenesis. Hereditas **21**: 1–112.

——. 1935*b*. The importance of apomicts for plant geography. Bot. Not. (Lund) **1935**: 325–330.

——. 1937. The occurrence of a sexual population within the apomictic *Taraxacum vulgare* group. Bot. Not. (Lund) **1937**: 332–336.

——. 1938*a*. The cytological differentiation of male and female organs in parthenogenetic species. Biol. Zentralbl. **58**: 608–616.

——. 1938*b*. A general theory for the interrelation of meiosis and mitosis. Hereditas **25**: 31–32.

——. 1939*a*. Differential polyploidy within the blackberries. Hereditas **25**: 33–47.

——. 1939*b*. The interrelation of meiosis and mitosis. I. The mechanism of agamospermy. Hereditas **25**: 289–322.

HABERLANDT, G. 1923*a*. Zur Embryologie von *Allium odorum.* Ber. Deut. Bot. Ges. **41**: 174–179.

——. 1923*b*. Über die Ursache des Ausbleibens der Reduktionsteilung in den Samenanlagen einiger parthenogenetischen Angiospermen. Sitzb. K. Preuss. Akad. Wiss. **1923**: 283–294.

HODGSON, R. W., AND CAMERON, S. H. 1938. Effects of reproduction by nucellar embryony on clonal characteristics of *Citrus.* Jour. Hered. **29**: 417–419.

HOLLINGSHEAD, L., AND BABCOCK, E. B. 1930. Chromosomes and phylogeny in *Crepis.* Univ. Calif. Publ. Agr. Sci. **6**: 1–53.

HOLMGREN, I. 1919. Zytologische Studien über die Fortpflanzung bei den Gattungen Erigeron und Eupatorium. K. Svensk. Vet. Akad. Handl. **59**(7): 1–118.

HORN, C. L. 1940. Existence of only one variety of cultivated mangosteen explalined by asexually formed "seed." Science **92**: 237–238.

JEFFREY, E. C., AND HAERTL, E. J. 1939*a*. The production of unfertilized seeds in *Trillium.* Science **90**: 81–82.

——, AND ——. 1939*b*. Apomixis in *Trillium.* La Cellule **48**: 77–88.

JOHNSON, A. M. 1936. Polyembryony in *Eugenia Hookeri*. Am. Jour. Bot. 23: 83–85.
JULIANO, J. B. 1934. Origin of embryos in the strawberry mango. Philip. Jour. Sci. 54: 553–556.
———. 1937. Embryos of Carabao Mango, *Mangifera indica* L. Philip. Agr. 25: 749–760.
———, AND CUEVAS, N. L. 1932. Floral morphology of the mango (*Mangifera indica* L.) with special reference to the pico variety from the Philippines. Philip. Agr. 21: 449–472.
KIELLANDER, C. L. 1935. Apomixis bei *Poa serotina*. Bot. Not. (Lund) 1935: 87–95.
———. 1937. On the embryological basis of apomixis in *Poa palustris* L. Svensk Bot. Tidskr. 31: 425–429.
KOROLEVA, V. A. 1939. Interspecific hybridization in the genus *Taraxacum*. Compt. Rend. (Doklady) Acad. Sci. URSS 24: 174–176.
KOSTOFF, D., AND TIBER, E. 1939. A tetraploid rubber plant, *Taraxacum kok-saghyz*, obtained by colchicine treatment. Compt. Rend. Acad. Sci. URSS 22: 119–120.
KUHN, E. 1928. Zur Zytologie von *Thalictrum*. Jahrb. Wiss. Bot. 68: 382–430.
LEVAN, A. 1940. The cytology of *Allium amplectens* and the occurrence in nature of its asynapsis. Hereditas 26: 353–394.
LIDFORSS, B. 1914. Résumé seiner Arbeiten in *Rubus*. Zeits. Ind. Abst. Vererb. 12: 1–13.
LILJEFORS, A. 1934. Über normale und apospore Embryoentwicklung in der Gattung *Sorbus*, nebst einigen Bemerkungen über die Chromosomenzahlen. Svensk Bot. Tidskr. 28: 290–299.
MARKLUND, G. 1938. Die *Taraxacum*-flora Estlands. Act. Bot. Fenn. 23: 1–150.
MARSDEN-JONES, E. M., AND TURRILL, W. B. 1935. Studies in Ranunculus. III. Further experiments concerning sex in *Ranunculus acris*. Jour. Genet. 31: 363–378.
MCDONALD, C. C. 1927. A study of seed development in three species of *Erigeron*, with special reference to apogamy. Bull. Torrey Bot. Club 54: 479–497.
METCALFE, C. R. 1939. The sexual reproduction of *Ranunculus Ficaria*. Ann. Bot. 3: 91–103.
MODILEWSKI, J. 1930. Neue Beitrage zur Polyembryonie von *Allium odorum*. Ber. Deut. Bot. Ges. 48: 285–294.
MUNTZING, A. 1928. Pseudogamie in der Gattung *Potentilla*. Hereditas 11: 267–283.
———. 1931. Note on the cytology of some apomictic *Potentilla* species. Hereditas 15: 166–178.
———. 1933. Apomictic and sexual seed formation in *Poa*. Hereditas 17: 131–154.
———. 1940. Further studies on apomixis and sexuality in *Poa*. Hereditas 26: 115–190.
———, TEDIN, O., AND TURESSON, G. 1931. Field studies and experimental methods in taxonomy. Hereditas 15: 1–12.
NOACK, K. L. 1939. Über *Hypericum*—Kreuzungen VI. Fortpflänzungsverhältnisse und Bastarde von *Hypericum perforatum* L. Zeit. Ind. Abst. Vererb. 76: 569–601.
OEHLER, E. 1927. Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta 3: 641–733.
OEHLKERS, F. 1937. Neue Versuche über zytologisch-genetische Probleme (Physiologie der Meiosis). Biol. Zentralbl. 57: 126–149.
OKA, H. T. 1935. Zytologische Beobachtungen über eine Mutant-sippe von *Erigeron annuus*. Jap. Jour. Genet. 10: 237–241. [Japanese. German

Okabe, S. 1930. Über Parthenogenesis bei *Houttuynia cordata*. Jap. Jour. Genet. 6: 14–19. [Japanese, German summary.]

————. 1932. Parthenogenesis bei *Ixeris dentata*. Bot. Mag. Tokyo 46: 518–523. [Japanese, German summary.]

Ostenfeld, C. H. 1910. Further studies on the apogamy and hybridization of the *Hieracia*. Zeits. Ind. Abst. Vererb. 3: 241–285.

————. 1921. Some experiments on the origin of new forms in the genus *Hieracium*, subgenus *Archieracium*. Jour. Genet. 11: 117–122.

Pace, L. M. 1913. Apogamy in *Atamosco*. Bot. Gaz. 56: 376–394.

Petrov, D. F. 1939. On the occurrence of facultative pseudogamy in a triploid variety of raspberries, Immer Tragende (*R. idaeus*). Compt. Rend. Acad. Sci. URSS 22. 352–353.

Pijl, L. V. D. 1934. Über Polyembryonie bei *Eugenia*. Rec. Trav. Bot. Néerl. 31: 113–187.

Poddubnaja-Arnoldi, V. A. 1933. Geschlechtliche und ungeschlechtliche Fortpflanzung bei einigen *Chondrilla*-Arten. Planta 19: 46–86.

————. 1939. Development of pollen and embryosac in interspecific hybrids of *Taraxacum*. Compt. Rend. Acad. Sci. URSS 24: 374–377.

————, and Dianova, V. 1934. Eine zyto-embryologische Untersuchung einiger Arten der Gattung *Taraxacum*. Planta 23: 19–46.

————, and ————. 1937. Character of propagation in some species of the genus *Taraxacum*. Bot. Zhurn. (USSR) 22: 267–295. [Russian, brief English summary.]

Popoff, A. 1935. Über die Fortpflanzungsverhältnisse der Gattung *Potentilla*. Planta 24: 510–522.

Redinger, K. 1938. Über die Entstehung diploider Embryonen aus unbefruchteten, mit gattungsfremden Pollen bestaübten Samenanlagen bei *Petunia nyctaginiflora*. Biol. Zentralbl. 58: 142–151.

Rosenberg, O. 1917. Die Reduktionsteilung und ihre Degeneration in *Hieracium*. Svensk Bot. Tidskr. 11: 145–206.

————. 1927. Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen. Hereditas 8: 305–338.

————. 1930. Apogamie und Parthenogenesis bei Pflanzen. Handb. Vererb. II.

Sato, D. 1938. Karyotype alteration and phylogeny. IV. Karyotypes in Amaryllidaceae with special reference to the SAT chromosome. Cytologia 9: 203–242.

Sax, H. J., and Sax, K. 1935. Chromosome structure and behavior in mitosis and meiosis. Jour. Arn. Arb. 16: 423–439.

Schnarf, K. 1929. Embryologie der Angiospermen. Handb. Pflanzenanat. 10(2) pts. 1–5.

Sharp, L. J. 1934. Introduction to cytology.

Sprecher, M. A. 1919. Étude sur la sémence et la germination du *Garcinia mangostana* L. Rev. Gen. Bot. 31: 513–531; 611–634.

Stebbins, G. L., Jr. 1932*a*. Cytology of *Antennaria* I. Normal species. Bot. Gaz. 94: 134–151.

————. 1932*b*. Cytology of *Antennaria* II. Parthenogenetic species. Bot. Gaz. 94: 322–345.

————. 1935. A new species of *Antennaria* from the Appalachian region. Rhodora 37: 229–237.

————, and Babcock, E. B. 1939. The effect of polyploidy and apomixis on the evolution of species in *Crepis*. Jour. Hered. 30: 519–530.

————, and Jenkins, J. A. 1939. Aposporic development in the North American species of *Crepis*. Genetica 21: 1–34.

Steil, W. N. 1939. Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. 5: 433–453.

STENAR, H. 1935. Parthenogenesis in der Gattung *Calamagrostis*. Ark. Bot. 25: 1–8.
SUESSENGUTH, K. 1923. Über die Pseudogamie bei *Zygopetalum Mackayi* Hook. Ber. Deut. Bot. Ges. 41: 16–23.
SUKATSCHEW, W. 1928. Einige experimentelle Untersuchungen über den Kampf ums Dasein zwischen Biotypen derselben Art. Zeits. Ind. Abst. Vererb. 47: 54–74.
SWINGLE, W. T. 1932. Recapitulation of seedling characters by nucellar buds developing in the embryo-sac of *Citrus*. Proc. 6th Int. Cong. Genet. 2: 196–197.
TACKHOLM, G. 1922. Zytologische Studien über die Gattung *Rosa*. Acta Horti. Bergiani 7: 97–381.
THOMAS, P. T. 1940. Reproductive versatility in *Rubus*. II. The chromosomes and development. Jour. Genet. 40: 119–128.
TINNEY, F. W. 1940. Cytology of parthenogenesis in *Poa pratensis*. Jour. Agr. Res. 60: 351–360.
——————, AND AAMODT, G. S. The progeny test as a measure of the types of seed development in *Poa pratensis* L. Jour. Hered. 31: 457–464.
TURESSON, G. 1926. Studien über *Festuca ovina* I. Normalgeschlechtliche, halb- und ganzvivipäre Typen nordischer Herkunft. Hereditas 8: 161–206.
——————. 1930. Studien über *Festuca ovina* II. Chromosomenzahl und Viviparie. Hereditas 13: 177–184.
——————. 1931. Studien über *Festuca ovina* III. Weitere Beiträge zur Kenntnis der Chromosomenzahlen viviparen Formen. Hereditas 15: 13–16.
TURRILL, W. B. 1938*a*. Problems of British *Taraxaca*. Proc. Linn. Soc. Lond. 150: 120–124.
——————. 1938*b*. Material for a study of taxonomic problems in *Taraxacum*. Rep. Bot. Soc. Exch. Club 11(1937): 570–589.
VAARAMO, A. 1939. Cytological studies on some Finnish species and hybrids of the genus *Rubus* L. Maataloustiet Aikak. (Agr. Mag. Helsingfors) 11: 72–85.
WEBBER, H. J. 1931. The economic importance of apogamy in *Citrus* and *Mangifera*. Proc. Am. Soc. Hort. Sci. 28: 57–61.
——————. 1932. Variations in *Citrus* seedlings and their relation to rootstock selection. Hilgardia 7: 1–79.
WEBBER, J. M. 1940. Polyembryony. Bot. Rev. 6: 575–598.
WEBER, E. 1929. Entwicklungsgeschichtliche Untersuchungen über die Gattung *Allium*. Bot. Arch. 25: 1–44.
WETTSTEIN, F. v. 1928. Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage II. Bibl. Genet. 10: 1–216.
WIGER, J. 1930. Ein neue Fall von autonomer Nucellarpolyembryonie. Bot. Not. (Lund) 1930: 368–370.
WINKLER, H. 1908. Über Parthenogenesis und Apogamie im Pflanzenreich. Prog. Rei. Bot. 2: 293–454.
——————. 1920. Verbreitung und Ursache der Parthenogenesis im Pflanzen- und Tierreiche.
——————. 1934. Fortpflanzung der Gewächse 7. Apomixis. Handw. Naturwiss. 4: 451–461.
WOODWORTH, R. H. 1930. Cytological studies in the Betulaceae. III. Parthenogenesis and polyembryony in *Alnus rugosa*. Bot. Gaz. 89: 402–409.
ZÜRN, K. 1939. Untersuchungen zur Physiologie der Meiosis. X. Neue Beiträge zur Kenntnis des Einflusses der Plastiden auf den Ablauf der Meiosis. Zeits. Bot. 34: 273–310.
ZWEIFEL, R. 1939. Cytologisch-embryologische Untersuchungen an *Balanophora abbreviata* Blume und *Balanophora indica* Wall. Viertel-

RESISTANCE OF PLANTS TO INSECT ATT

RALPH O. SNELLING[2]
Bureau of Plant Industry

INTRODUCTION

The word "resistance" is used here in its widest and perhaps linguistically incorrect sense to include those characteristics which enable a plant to avoid, tolerate or recover from the attacks of insects under conditions that would cause greater injury to other plants of the same species. This is, however, the usage employed in much recent entomological literature.

The choice of varieties with reference to their ability to produce satisfactory yields in the presence of insect infestations has been recognized during a period of about one and one-half centuries. As early as 1788, Isaac Chapman (82) recognized and advocated the cultivation of wheat varieties having an early growth habit as a means of reducing damage caused by the Hessian fly (*Phytophaga destructor* Say). George Lindley (321) in 1831 recommended the Winter Majetin and Siberian Bitter-Sweet varieties of apple because of their resistance to the serious apple pest, woolly aphid (*Eriosoma lanigerum* Hausm.), in England. The grape Phylloxera (*Phylloxera vitifoliae* Fitch) was discovered on an American vine in 1854 by Asa Fitch (53), and in 1869 (170) the high degree of resistance of American vines to this insect was discovered. Since these pioneer observations many similar examples have been recorded in the literature.

Insect resistance in plants and related subjects has been reviewed and discussed by several individuals. Many of the papers and books listed below include selected bibliographies. Brues (61, 62, 63), Felt and Bromley (167), Flint and Bigger (180), Forbes (187), Graham (199), Hunter and Leake (265), Imms (275), Lees (318), MacLeod (323), Martin (339), McColloch (352), Mumford (382),

[1] Cooperative investigation of the U. S. Department of Agriculture, Illinois Agricultural Experiment Station and Illinois Natural History Survey.

[2] Appreciation is expressed to the following men for helpful suggestions in the preparation of this review: N. E. Stevens, University of Illinois; M. T. Jenkins and J. H. Martin, Bureau of Plant Industry, U. S. Department of Agriculture; Reginald H. Painter, Kansas State College; J. H. Bigger, Illinois Natural History Survey; C. M. Packard, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture; and S. F. Bailey, University of California.

STENAR, H. Br. and Hey (383), Painter (397), Parker and Painter SUESSEN Sweetman (501), Treherne (518), Wardle (543), and SUKA ..e and Buckle (544).

.he oldest published record of plant resistance reported in this review is that by Havens (229) in 1792 in which he recognized the Hessian fly resistance of the Underhill variety of wheat. After the appearance of his paper there was a considerable period in which little attention was given to insect resistance in plants. Only 37 papers reporting resistance were published during the 128-year period, 1792–1920. During the 10-year period that followed, more interest was shown in the study of plants resistant to insect attack, and a total of 57 papers recording plant varieties resistant to attack were published. In the early part of the decade, 1931–1940, increasing interest was shown, but there was still a tendency among research workers to minimize the importance of host resistance, and some even denied its possibility. A total of 163 papers reporting plant varieties resistant to insect attack were published during this decade.

It is plainly evident from the general publications just cited and those presented in the tables that follow, and from the recent programs arranged by various entomological societies, that interest in host resistance has greatly increased. Indeed, it seems at the present time that there is danger of going to the extreme in expecting too much from breeding plants for insect resistance. It is realized that the methods of controlling certain plant-feeding insects through use of insecticides and cultural practices, such as crop rotation and sanitation, have their limitations. But these methods all have and will continue to have their place in agriculture. Likewise, breeding varieties of crops resistant to insect attack has its limitations, and in many cases the results may be only temporary attainments. The length of time that a resistant variety will remain resistant is a matter of speculation, but certainly the occurrence of biological strains of insects offers a reason for caution in expecting too much from breeding resistant varieties.

RECORDS AND IMPORTANCE OF HOST RESISTANCE

The problems of plant resistance and the repellence of insect attacks, as well as tolerance to and evasion from attack, have attracted much attention in recent years. Available literature includes

records of resistance in nearly 100 plant species, and over 100 insect species are involved. These are summarized in table 1.

The development of immune varieties of crop plants that are equal in yielding ability and quality to those now in use would be one of the most economical control measures. However, immune varieties are extremely rare, and what is called resistance often implies little more than a low degree of susceptibility to attack. That is, resistance, as used in some of the literature, may be so high as to confer practical immunity or may be so low as to merely a degree of susceptibility.

The literature indicates that resistance to one insect does not usually imply resistance to other insect species, and in some cases a variety that is resistant to one biological form of an insect species is highly susceptible to another form. It is shown, further, that the factors which control or influence resistance or susceptibility are varied, and that the expression of the genetic factors responsible for resistance is often modified by environmental conditions. However, in mentioning a few of the conditions responsible for the complex nature of host resistance to insects, it is not intended that the importance of this field of research be minimized, or that it be inferred that host resistance is the only important insect control measure. It does have sufficient promise to warrant attention in connection with the general field of insect control, and deserves consideration in any plant breeding program. In many cases it is necessary to select strains of plants that have a fair degree of resistance combined with many other desirable characteristics in preference to those that have the highest possible resistance. Other effective control measures should also be considered in many cases can be used to advantage in conjunction with resistant varieties.

PLANT CHARACTERISTICS SUGGESTED AS HAVING AN INFLUENCE IN RESISTING INSECT ATTACK

It is probable that in most cases no single plant character is responsible for a variety surviving insect attack. The plant characteristics that have been suggested as having an influence upon insect resistance are so numerous and vary so widely that a satisfactory classification is difficult. They have been classified in some of the literature under four headings: physical, chemical, physiological and a combination of physical, chemical and physiological. In table 2

TABLE 1

RECORDS OF INSECT RESISTANCE[3]

Host	Insect
Abaca (*Musa textilis*)	Slug caterpillar, *Thosea sinensis* Wlk. (473)
Alfalfa	Potato leafhopper, *Empoasca fabae* (Harr.) (286)
	Pea aphid, *Illinoia pisi* (Kalt.), (50, 123, 137, 158, 401)
Apple	Woolly apple aphid, *Eriosoma lanigerum* (Hausm.) (94, 321, 345, 454, 494, 530, 532)
	Apple leafhopper, *Empoasca mali* LeB. (195, 462, 549)
	Codling moth, *Carpocapsa pomonella* L. (119, 166, 280, 386)
	Apple moth, *Hyponomeuta padellus* L. (328, 492)
	Leaf-curling midge, *Dasyneura mali* Kieffer (552)
	Apple fruit fly, *Rhagoletis pomonella* (Walsh) (144, 427)
	Cranberry rootworm, *Rhabdopterus picipes* (Oliv.) (218)
	Plum curculio, *Conotrachelus nenuphar* (Herbst.) (198)
	Green fruit worm, *Graptolitha antennata* (Walk.) (492)
Bamboo	Powder-post borer, *Dinoderus minutus* (F.) (13)
Basket willows (*Salix* sp.)	Gall midges, *Rhabdophaga heterobia* H. Lw., *Rhabdophaga terminalis* H. Lw., *Rhabdophaga saliciperda* Duf. (29, 30, 31)
Beans (*Phaseolus* sp.)	Potato leafhopper, *Empoasca fabae* (Harr.) (17, 42)
	Lygus plant bugs, *Lygus elisus* Van Duzee, *Lygus hesperus* Knight (472)
	Pod borers (species not indicated) (20)
	Mexican bean beetle, *Epilachna varivestis* Muls. (324)
	Thrips sp. (303)
	Weevil (species not indicated) (474)
	Melon fly, *Chaetodacus cucurbitae* (Coq.) (252)
Beans, broad (*Vicia narbonensis*)	*Aphis rumicis* L. (130)
Blackberry	Red-necked cane borer, *Agrilus ruficollis* F. (245)
	Japanese beetle, *Popillia japonica* New. (175)
Cabbage	Cabbage root maggot, *Hylemyia brassicae* (Bouche) (326)
Celery	Tarnish plant bug, *Lygus pratensis* (L.) (323)

[3] An attempt has been made to use the scientific name of the insect that was used in the publications reviewed. The only exception is the corn ear worm. This insect has been discussed under the names *Heliothis obsoleta* Fitch. and *H. armigera* Hb., and since the latter is now the accepted specific name, it has been used in this review, although most papers have used *H. obsoleta* Fitch.

TABLE 1.—(*Continued*)

Host	Insect
Cherry	Peach and prune root borer, *Synanthedon opalescens* H. Edw. (510)
Chrysanthemum	Chrysanthemum midge, *Diarthronomyia hypogaea* F. Lw. (210)
Clover, Berseem	Clover seed chalcid, *Bruchophagus gibbus* (Boh.) (302)
Clover, Red	Potato leafhopper, *Empoasca fabae* (Harr.) (286, 287, 288, 373, 432, 433)
Coffee	Coffee berry beetle borer, *Stephanoderes hampei* Ferr. (102)
Cotton	Boll weevil, *Anthonomus grandis* Boh. (97, 98, 99, 153, 266, 268, 545)
	Leaf blister mite, *Eriophyes gossypii* Banks (213)
	Black scale, *Saissetia nigra* Nietn. (214)
	Cotton jassid, *Chlorita fascialis* Jacobi. (372, 411, 412, 564, 565)
	Cotton stainer, *Dysdercus superstitiosus* F. (343)
	Pink boll worm, *Pectinophora gossypiella* Saun. (81, 561, 562)
	Cotton flea-hopper, *Psallus seriatus* Reut. (507, 508)
	Thrips, *Sericothrips variabilis* Beach (546, 547)
Cowpea	Weevils (species not indicated) (474)
	Cowpea curculio, *Chalcodermus aeneus* Boh. (11, 12)
Cranberry	Blunt-nosed leafhopper, *Ophiola striatula* Fall. (553)
Currant	Currant mite, *Eriophyes ribis* Nale. (317)
Dewberry	Red-necked cane borer, *Agrilus ruficollis* F. (245)
	Japanese beetle, *Popillia japonica* New. (175)
Filbert	*Melissopus latiferreanus* (Walsi.) (147)
Gladiolus	Gladiolus thrips, *Taeniothrips gladioli* M. & S. (*T. simplex* Morison) (18, 241)
Gooseberry	Gooseberry witch-broom aphid, *Myzus houghtonensis* (Troop) (139)
Grape	Grape Phylloxera, *Phylloxera vitifoliae* Fitch (47, 48, 52, 271, 528)
	Grape-vine thrips, *Phipiphorothrips cruentetus* Hd. (437)
Grapefruit	Mediterranean fruit fly, *Ceratitis capitata* Wied. (298)
Grasses (Many species)	Chinch bug, *Blissus leucopterus* (Say) (235)
	Hairy chinch bug, *Blissus hirtus* Montadon (325)
	Blue grass webworm, *Crambus teterellus* Zinck (388)
	Hessian fly, *Phytophaga destructor* (Say) (291, 293, 294)
Greens (Mustard-Spinach and Japanese Turnip or Shogoin)	Aphids (species not indicated) (446)
Lilac	Lilac leaf-miner, *Gracilaria syringella* F. (272)

TABLE 1.—(*Continued*)

Host	Insect
Maize	Corn ear worm, *Heliothis armigera* Hb. (49, 67, 145, 146, 231, 238, 330, 349, 399, 400, 429, 430, 431, 539)
	Rice weevil, *Sitophilus oryzae* L. (77, 78, 79, 243)
	Leaf aphid, *Aphis maidis* Fitch (103, 350, 485, 541)
	Root aphid, *Aphis maidis-radicus* Forbes (193)
	Chinch bug, *Blissus leucopterus* (Say) (150, 179, 184, 181, 250, 404, 406, 486)
	European corn borer, *Pyrausta nubilalis* Hb. (159, 160, 172, 182, 183, 225, 333, 336, 337, 338, 366, 419, 448, 450, 456, 460, 511, 512, 536)
	Southern corn rootworm, *Diabrotica duodecimpunctata* Fab. (46, 101, 150, 151)
	Grasshoppers, *Melanoplus* species (64, 263, 264)
Oats	Frit fly, *Oscinella frit* L. (113, 114, 116, 190, 219)
	Wireworms (several species) (340)
Onion	Thrips, *Thrips tabaci* Lind. (296. 297, 323, 348, 476, 500, 538)
	Onion maggot, *Hylemyia antiqua* Meig. (475)
Orange	Mediterranean fruit fly, *Ceratitis capitata* Wied. (298)
Papaya (*Carica papaya*)	Papaya fruit fly, *Toxotrypana curvicauda* Gerst. (341)
Peach	Oriental fruit worm, *Laspeyresia molesta* Busck. (496)
Peas, Canning	Pea aphid, *Illinoia pisi* (Kalt.) (329, 468)
Pecan	*Phylloxera devastatrix* Perg. (21)
Potato	Wireworms (several species) (367)
	Psyllid, *Paratrizoa cockerilli* Sulc. (224)
	Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (459, 519, 520, 522)
	Potato leafhopper, *Empoasca fabae* (Harr.) (23, 477, 479)
	Potato flea beetle, *Epitrix subcrinita* LeConte (211, 324)
Raspberry	Raspberry aphid, *Amphorophora rubi* Kalt. (463, 558)
Rice	Leafhoppers, *Nephotettix bipunctalus* Fab., *N. apicalis* Matsch (369)
	Stem borers, *Schoenobius incertillus* Wlk., *Chilo simplex* Bult. (471)
Sorghum	Sorghum midge, *Contarinia sorghicola* Coq. (22)
	Chinch bug, *Blissus leucopterus* (Say) (406, 408, 486, 487)
	Grasshoppers, *Melanoplus* species (64)
Soybean	Potato leafhopper, *Empoasca fabae* (Harr.) (290, 373, 433)
	Grapholitha glycinivorella Mats. (384)
Squash	Squash bug, *Anasa tristis* (Deg.) (249)
Strawberry	Strawberry aphid, *Capitophorus fragariae* (129, 491)
	Strawberry weevil, *Anthonomus signatus* Say (15)

TABLE 1.—(*Continued*)

Host	Insect
Sugar beet	Sugar beet leafhopper, *Eutettix tenella* Baker (10, 74, 148, 161)
Sugar cane	Sugar cane leafhopper, *Perkinsiella saccharicida* Kirk. (533)
	Moth borer, *Diatraea saccharalis* F. (56, 92, 141, 223, 526, 527, 560)
	Sugar cane froghopper, *Monecphora saccharina* Dist. (168, 559)
	Sugar cane aphid, *Aphis sacchari* Zehnt. (422)
	Sugar cane beetle borer, *Rhabdocnemis obscura* Boisd. (422)
	Lachnosterna smithii Arrow (285)
	Locust species (196)
	Sugar cane grub, *Lepidoderma albohirtum* (36, 37, 39)
Sunflower	Sunflower moth, *Homoeosoma nebulella* Hb. (451)
Tobacco	Potato flea beetle, *Epitrix subcrinita* LeConte (377)
Tomato	Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (312)
	Melon fly, *Chaetodacus cucurbitae* (Coq.) (332)
Trees	*Termite* species (33, 207, 391, 488, 489)
(Many species)	Gipsy moth, *Porthetria dispar* L. (66, 208, 378)
	Brown-tail moth, *Euproctis chrysorrhoea* L. (66)
	Chrysomphalus dictyospermi (Morg.) (109)
	Bark beetle, *Dendroctonus brevicomis* Lec. (299)
	Locust borer, *Cyllene robiniae* Forst. (209)
	Dendrolimus pini L. (359)
Turnip	Turnip maggot (species not indicated) (163)
Vetch	Pea aphid, *Illinoia pisi* Kalt. (2)
Walnut, Persian (*Juglans regia*)	Walnut husk fly, *Rhagoletis completa* Cress. (57, 206)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (85, 86, 140, 174, 188, 227, 229, 351, 353, 392, 393, 403, 444, 455, 531, 563)
	Stem maggot, *Meromyza americana* Fitch (3, 43, 152, 548)
	Chinch bug, *Blissus leucopterus* (Say) (292)
	Frit fly, *Oscinella frit* L. (84)
	Wireworms (several species) (340, 499)
	Sawfly (301)

fifteen categories are used, leaving to the reader the choice of a more general classification if any is desired. Certain classes include a much greater number of examples than other, but this is merely what the literature shows, and in the opinion of the writer it has no relation to the biological significance of any particular class.

A study of the plant characteristics responsible for insect resistance has importance to the plant breeder who attempts to differentiate the

several genetic factors that may be involved. A knowledge of these characteristics is also of importance to the entomologist who is concerned with insect life history studies. There are numerous cases in which the variety of plant has had a marked effect on insect development. Many papers dealing with insect biology fail to mention the species or variety of plant used in the rearing studies. The influence of the variety upon insect development has no doubt been partially responsible for differences that have been reported on the biology of certain insects. It is apparent that more attention is being given to the influence of plant varieties upon insect development, and a few examples are presented here which should emphasize the importance of this factor in biological studies.

Dahms *et al.* (126, 127) and Dahms and Martin (124) have shown that the variety of sorghum has a very marked effect on the biology of the chinch bug (*Blissus leucopterus* (Say)). In one experiment, ten females were fed on plants of Dwarf Yellow milo, a susceptible variety, and the same number was fed on Atlas sorgo, a resistant variety. The average life of the females fed on the susceptible variety was 40 days, and during this period a total of 1179 eggs were oviposited. Those fed on Atlas sorgo, the resistant variety, had an average life of 15 days, and during this period only 9 eggs were oviposited by the ten females. It was also shown that variety had a marked influence on the development of immature stages of the chinch bug. An average of 35 days was required for the nymphs to develop from hatching to adult when fed on the susceptible Dwarf Yellow milo plants, while those fed on the resistant Atlas sorgo plants required an average of 45 days. Only 14% of the nymphs fed on the resistant variety reached the adult stage while 92% of those fed on the susceptible variety reached the adult stage.

Dahms and Painter (123) found that pea aphids (*Illinoia pisi* (Kalt.)) reproduced more rapidly, and the mortality was less when fed on alfalfa plants that appeared susceptible in the field than when fed on those that appeared resistant. The aphids were also found to reproduce more rapidly on selfed progeny of susceptible plants than on selfed progeny of resistant plants. It was also shown that the aphids reproduced more rapidly when fed on flowering branches than when fed on vegetative branches. This latter observation is somewhat similar to that made by Painter (396) while working with Hessian fly (*Phytophaga destructor* Say). By

following the individual history of eggs of Hessian fly laid on different leaves of the wheat plant, it was shown that no flaxseed survived from eggs laid on the outer or first leaf; 6.4% survived from eggs laid on the second leaf; and 45.4% survived from those laid on the third or central leaf. Painter *et al.* (403) have also shown that Hessian fly flaxseed and larvae maturing from eggs laid by the same female are much larger and stronger when allowed to develop on Kanred wheat than when allowed to develop on Blackhull.

Davidson (130) studied the reproduction of *Aphis rumicis* that were feeding on 18 varieties of field beans. From the results obtained, the varieties were rated from 3% to 98% susceptible. Winter (558) stated that Herbert raspberry exhibits a very marked resistance to *Amphorophora rubi* Kalt. Data are presented which indicate that under favorable conditions, with its insect enemies removed, *A. rubi* will feed and live on the Herbert variety for several weeks but is unable to maintain its population. The population on check plants of other varieties invariably increased, often at a rapid rate. Huber and Schwartze (258), working with the same insect, show that on certain varieties *A. rubi* failed to reproduce, while on others reproduction was slow and the population remained small. It was shown that the aphids fed and reproduced abundantly on susceptible varieties. Searls (468) reports similar results with pea aphids feeding on different varieties of canning peas, and Roach (442) and Roach and Massee (443) found that different varieties of apples had a marked influence upon the reproduction rate and development of the woolly aphid (*Eriosoma lanigerum* Hausm.).

Crop plants frequently thrive over a greater range of temperatures than can the insects which attack them, and for this reason certain varieties have in some cases been considered to be resistant. This, of course, is not a case of resistance in the plants but is an example of evasion. However, the importance of this condition should be recognized as of value in conjunction with resistance and other insect control measures. The maturity of a variety which may be influenced by either ecologic or genetic factors has been recognized as an important factor in reducing insect damage. The use of early maturing varieties of cotton and early planting dates has been recommended as means of overcoming boll weevil injury. Early ripening varieties of rice have been recommended in India because they suffer less damage from leafhoppers than later maturing

varieties. A number of other crop plants are known to evade insect damage because of late maturity. Late maturing varieties of maize or early maturing varieties planted late in the season are injured less by the European corn borer than those varieties that are farther advanced toward maturity at the time of moth flight.

Certain varieties of crop plants are known to be unattractive to the insect. This condition may result in a smaller number of insects feeding on a variety and in some cases a smaller number of eggs being deposited on the variety. The cabbage butterfly is attracted only to varieties having a particular glucoside which is one of the mustard oils. Varieties lacking this glucoside usually are not attacked. The coloration of the panicles at the flowering stage of certain grasses has been suggested as causing some strains to be more readily attacked by the cocks-foot moth. Varieties having green to pale yellow color suffered less damage than those having a steel blue tinge. The blue color was thought to influence the moths in making their choice of plants for oviposition. The western pine bettle is believed to be attracted to certain trees because of the formation and escape of volatile aldehydes or esters which are by-products of a respiratory fermentation resulting from abnormal enzyme activity in subnormal trees. Normal healthy trees are less attractive to the beetles. While the resistance of some varieties is dependent upon the absence of certain substances or conditions attractive to the insects that attack them, others are resistant because of the presence of substances that repel the insects. This is best illustrated by the resistance of certain trees to termites. In this case the insect is repelled from trees possessing certain volatile constituents. Several substances seem to be of importance, and it has been determined that guajol, sesquiterpene alcohol, camphor green oil and phenol have an important influence in repelling termites from trees possessing these substances. Resistance to termites does not seem to be in any way associated with the hardness of the wood. However, with certain other insects hardness of tissue seems to have a very marked influence on the resistance of a variety. The cherry fruit flies are unable to oviposit their eggs in cherries that possess a certain degree of hardness. This may be influenced by either the stage of maturity of the fruit or varietal characteristics. Soft-wooded chrysanthemums have been reported to be more susceptible to *Paroxyna misella* Lw. than the hard-wooded varieties. Sugar cane varieties have

been found resistant to several insect pests because of hardness of tissue in various parts of the plants.

The pubescence on the stems and leaves of certain varieties of alfalfa, apple, clover, cotton and soybeans has been shown to be of importance in connection with leafhopper resistance. Little is known concerning the exact manner in which pubescence affects the insect but it is known that the presence of pubescence discourages both feeding and oviposition.

Thickness of certain plant tissues has been shown to have an important bearing on the resistance of plants to certain insects. This characteristic may prevent feeding or satisfactory oviposition. The resistance of certain bean varieties to *Lygus* plant bug attack appears to be entirely dependent upon the thickness of the pod. It has been shown that the pods of the Great Northern variety can be punctured at any stage of development, while other varieties such as Butternut, Giant Stringless, Bountiful and Brittle Wax can not be punctured to a depth where the bean is reached. The tendency toward a thicker epidermis and cuticle in resistant varieties of sugar beets indicates an external protection from leafhopper attack. The influence of thickness of tissue upon oviposition is illustrated by the inability of the papaya fruit fly to reach through the flesh of the fruit of certain strains of papaya and satisfactorily oviposit their eggs.

The resistance of certain plant tissue to puncturing has been shown to have a very definite influence in the resistance of certain plant varieties to sucking insects. The leaves of Kentucky red clover have been shown to be much more resistant to needle puncturing than the leaves of Wisconsin 4, Oregon 6 and Oregon 8. The Kentucky strain is also more resistant to leafhopper injury. Application of fertilizer to seedling cabbage plants has been shown to materially reduce wireworm damage. It was believed that the increased toughness of the stems of plants growing on the fertilized plots was directly responsible for the reduction in wireworm damage. The resistance of the rag of the rind in citrus fruit is known to prevent the larvae of the Mediterranean fruit fly from reaching the pulp of the fruit.

The rate at which some varieties grow and the type of growth have been suggested as having an influence on resistance. Strains of alfalfa that have a greater proportion of new growth are usually

more severely attacked by the pea aphid. The moth of the European corn borer prefers those varieties that are more advanced in growth at the time they oviposit their eggs. Thrip resistance in onions has been associated with the shape of the leaves, the angle of divergence of the two innermost leaves, and the distance between the leaf-blades on the sheath column. Varieties offering the least protection for the thrips are usually more resistant than those that offer considerable protection. Trees have been shown to vary considerable in pore or vessel size, and trees having a vessel size smaller than the eggs of the powder post beetle are free from attack. It is necessary for this insect to oviposit in the pores in order for an infestation to become established.

Certain insects seem to be able to feed on some varieties of crop plants but the food that is obtained does not seem to be satisfactory for normal development of the insect. Varieties of alfalfa, apple, beans, gooseberry and raspberry apparently are resistant to species of aphids that attack them because of an incompatible food relationship. Chinch bugs and European corn borer have been shown to develop very poorly on varieties that were considered resistant to attack. This also has been shown in the case of Hessian fly fed on resistant varieties of wheat.

A few examples have been recorded where a physiological response of the plant following attack has a bearing on varietal resistance. Proliferation of cotton following attack by the boll weevil is perhaps the outstanding example. The proliferous tissue is not toxic, but rather the larvae are destroyed in a mechanical way from simple pressure. Cotton varieties appear to differ regarding the proportion of cases in which weevil punctures stimulate proliferation.

Some varieties of a crop appear to be able to tolerate more insect feeding than other varieties. Very little information is available regarding the basic causes of this tolerance, but certain varieties of maize and sorghums are known to produce satisfactory yields while other varieties growing adjacent to them fail in the presence of approximately the same infestation. This condition has also been reported in connection with Hessian fly resistance in wheat.

The ability of some varieties to recover after attack is also of considerable importance in connection with resistance investigations, although it is not a case of actual resistance. Certain varieties of barley and oats have been found very useful in areas infested with

frit fly largely because they were able to recover and produce rather satisfactory yields after the frit fly infestation had subsided. The ability of certain strains of maize and sugar cane to reproduce roots following attacks by southern corn rootworm and white grub, respectively, has resulted in the recommendation that these varieties be grown under conditions where these insects are likely to be a problem.

The absence of vigor in maize has been shown to be associated with reduced damage caused by the European corn borer. But the presence of considerable vigor in maize and sorghums tends to reduce the amount of damage caused by chinch bugs.

The resistance of varieties of crop plants depends upon their inherent resistance and their adaptation to soil, climate, and other conditions of environment. The inherent resistance of some crop plants is modified considerably by changes in environment while in some other crops the effect is not so great. Varieties of rice that have proven resistant to leafhoppers when grown on clay loam and high lying soils with calcareous formations have been reported to be severely damaged when grown on fertile well manured soil. Certain varieties of tea have been shown to be resistant to *Helopeltis theivora* Waterh. when grown in soil with an optimum supply of moisture, but this resistance was reduced by excessive soil moisture or drouth. The high resistance of the American vine to *Phylloxera* is also greatly modified when the vines are grown under different soil and moisture conditions.

BREEDING PLANTS TO REDUCE INSECT DAMAGE

A sound knowledge of the factors conferring immunity or resistance and the conditions which influence these factors, is the basis of breeding plants for insect resistance. However, it is not necessary to understand thoroughly these factors in order to make progress in a breeding program. It is highly probable that even the earliest plant breeders were unconsciously selecting for resistance to insects in developing high-yielding crops of good quality. The ultimate value of a variety depends upon its ability to reach a mature stage and produce a satisfactory yield in spite of the insects, diseases and other adverse environmental factors that beset it.

Breeding cultivated plants for insect resistance does not necessarily involve a special breeding program. It is not fundamentally

TABLE 2

PLANT CHARACTERISTICS SUGGESTED AS HAVING AN INFLUENCE IN RESISTING INSECT ATTACK

	I. Early Maturity (genetic or ecological)
Cotton	Boll weevil, *Anthonomous grandis* Boh. (97, 98, 268, 269, 278)
	Pink boll worm, *Pectinophora gossypiella* Saun. (25, 498, 554)
Rice	Leafhoppers, *Nephotettix bipunctatus* Fab., *Nephotettix apicalis* Matsch. (369)
Soybeans	*Grapholitha glycinivorella* Mats. (384)
	II. Late Maturity (genetic or ecological)
Apple	Apple maggot, *Rhagoletis pomonella* (Walsh) (83)
	Codling moth, *Carpocapsa pomonella* L. (280)
Maize	European corn borer, *Pyrausta nubilalis* Hb. (136, 171, 300, 363, 364, 365, 366, 420)
Onion	Thrips, *Thrips tabaci* Lind. (323)
Strawberry	Weevil, *Anthonomus signatus* Say. (15)
Sugar cane	Moth borer, *Diatraea saccharalis* F. (560)
	Sugar cane grub, *Lepidoderma albohirtum* (36, 37)
	III. Unattractiveness (feeding or oviposition)
Cabbage	Cabbage butterfly, *Pieris rapae* L. (517, 534)
Cranberry	Blunt-nosed leafhopper, *Ophiola striatula* Fall. (553)
Coffee	Coffee berry beetle borer, *Stephanoderes hampei* Ferr. (102)
Cotton	*Platyedra gossypiella* Saund. (493)
Gooseberry	Gooseberry witch-broom aphid, *Myzus houghtonensis* (Troop) (139)
Grasses	Cocks-foot moth, *Glyphipteryx fischeriella* Zell. (88)
Maize	Leaf aphid, *Aphis maidis* Fitch. (186)
	Corn ear worm, *Heliothis armigera* Hb. (27, 349)
	Grasshoppers, *Melanoplus* sp. (64, 441)
Pine trees	Western pine beetle (423)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (26, 27, 233, 487)
	Grasshoppers, *Melanoplus* sp. (64)
Sugar Cane	Moth borer, *Diatraea saccharalis* F. (253, 525)
Tomato	Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (312)
Wheat	Chinch bug, *Blissus leucopterus* (Say) (292)
	Hessian fly, *Phytophaga destructor* (Say) (353)
	Stem maggot, *Meromyza americana* Fitch (194)
	IV. Repellence
Sugar cane	Moth borer, *Diatraea saccharalis* F. (253, 525)
Trees (Several species)	Termite sp. (207, 391, 489)
	V. Pubescence
Alfalfa	Leafhopper, *Empoasca fabae* (Harr.) (254, 286, 428)
Apple	Leafhopper, *Empoasca fabae* (Harr.) (462)

TABLE 2.—(*Continued*)

Clover	Leafhopper, *Empoasca fabae* (Harr.) (432)
Cotton	Jassid, *Chlorita fascialis* Jacobi (270, 411, 412, 414, 415, 564)
	Cotton aphid (155)
Soybean	Leafhoppers, *Empoasca fabae* (Harr.) (290)
	Anomala rufocuprea Matsch. (309)
	Grapholitha glycinivorella Mats. (390)
	VI. Hardness of Tissue
Apple	Codling moth, *Carpocapsa pomonella* L. (438)
Cherry	Cherry fruit flies, *Rhagoletis cingulata* Loew., *Rhagoletis fausta* Osten Sacken, *Rhagoletis pomonella* (Walsh) (164)
Chrysanthemum	*Paroxyna misella* Lw. (248)
Coffee	Coffee berry beetle borer, *Stephanoderes hampei* Ferr. (102)
Cotton	Boll weevil, *Anthonomous grandis* Boh. (153)
Grasses	Chinch bug, *Blissus leucopterus* (Say) (235)
(Many species)	Hessian fly, *Phytophaga destructor* (Say) (294)
Rubber tree (*Hevea brasiliensis*)	Boring beetles, *Xyleborus parvulus; Pterolophia melanura* (470)
Sugar cane	Moth borer, *Diatraea saccharalis* F. (38, 236, 237, 253, 525)
	Sugar cane grub, *Lepidoderma albohirtum* (36, 37)
	Beetle borer, *Rhabdocnemis obscura* Boisd. (69, 555, 556)
Sunflower	Sunflower moth, *Homoeosoma nebulella* Hb. (451)
Walnut, Persian (*Juglans regia*)	Walnut husk fly, *Rhagoletis completa* Cress (57, 58)
	VII. Thickness of Tissue
Beans (*Phaseolus* sp.)	Lygus plant bugs, *Lygus elisus* Van Duzee; *Lygus hesperus* Knight (472)
Broad bean (*Vicia narbonensis*)	*Aphis rumicus* L. (131, 132)
Cotton	Boll weevil, *Anthonomus grandis* Boh. (97, 98, 99, 269)
Oats	Frit fly, *Oscinella frit* L. (113)
Onion	Thrips, *Thrips tabaci* Lind. (297)
Papaya (*Carica papaya*)	Papaya fruit fly, *Toxotrypana curvicauda* Gerst (341)
Sugar beet	Leafhopper, *Eutettix tenella* (Baker) (381)
	VIII. Toughness of Tissue
Alfalfa	Leafhopper, *Empoasca fabae* (Harr.) (287, 288)
Cabbage	Wireworms, (several species) (203)
Clover	Leafhopper, *Empoasca fabae* (Harr.) (287, 288)
Citrus	Mediterranean fruit fly, *Ceratitis capitata* Wied. (298)
Maize	Leaf aphid, *Aphis maidis* Fitch (193)
Potato	Leafhopper, *Empoasca fabae* (Harr.) (4, 23, 169, 347, 479)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (408, 487)

TABLE 2.—(*Continued*)

Sugar cane	Moth borer, *Diatraea saccharalis* F. (93)
	Sugar cane top borer, *Scirpophaga nivella* F. (276)
	Froghopper, *Monecphora saccharina* Dist. (559)
	IX. Growth Habit (rate and type)
Apple	Leaf-curling midge, *Dasyneura mali* Kieffer (552)
	Apple moth, *Hyponomeuta padellus* L. (328)
Maize	Corn ear worm, *Heliothis armigera* Hb. (26, 77, 95, 145, 146, 313, 330, 349, 400, 424, 425, 426, 539)
Onion	Thrips, *Thrips tabaci* Lind. (297)
Sorghum	Stem borers, *Pyrausta nubilalis* Hb.; *Diatraea diatraea* (257)
Sugar cane	Wireworms (Several species) (371)
Trees	Powder post borer, *Lyctus brunneus* Stephens (89, 90, 91, 110, 409)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (82, 85, 188)
	X. Incompatible Food Relations
Alfalfa	Pea aphid, *Illinoia pisi* (Kalt.) (50)
Apple	Woolly aphid, *Eriosoma lanigerum* (Hausm.) (202, 374, 442)
Broad bean (*Vicia narbonensis*)	*Aphis rumicus* L. (131, 132)
Currant	Currant mite, *Eriophyes ribis* Nal. (317)
Gooseberry	Gooseberry witch-broom aphid, *Myzus houghtonensis* (139)
Maize	Chinch bug, *Blissus leucopterus* (Say) (127)
	European corn borer, *Pyrausta nubilalis* Hb. (55)
Oats	Frit fly, *Oscinella frit* L. (113)
Potato	Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (521, 523)
Raspberry	Raspberry aphid, *Amphorophora rubi* Kalt. (258, 463, 558)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (122, 126, 127, 487)
Strawberry	Weevil, *Anthonomus signatus* Say. (15)
Sugar beet	Leafhopper, *Eutettix tenella* (Baker) (75)
Sugar cane	Froghopper, *Monecphora saccharina* Dist. (559)
Tea	Tea mosquito bug, *Helopeltis theivora* Waterh. (6, 7)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (351, 354, 396, 403)
	XI. Physiological Response of Plant
Cotton	Boll weevil, *Anthonomous grandis* Boh. (242)
Citrus	Mediterranean fruit fly, *Ceratitis capitata* Wied. (14)
	XII. Tolerance to Attack
Cotton	Jassid, *Chlorita fascialis* Jacobi (411)
Maize	Chinch bug, *Blissus leucopterus* (Say) (179, 404, 486)

TABLE 2.—(*Continued*)

Sorghum	Chinch bug, *Blissus leucopterus* (Say) (487)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (403)
	XIII. Recovery Following Attack
Barley	Frit fly, *Oscinella frit* L. (310)
Grasses (Many species)	Chinch bug, *Blissus leucopterus* (Say) (235)
Maize	Southern corn rootworm, *Diabrotica duodecimpunctata* Fab. (46)
Oats	Frit fly, *Oscinella frit* L. (112, 113, 219)
Rice	Stem borers, *Schoenobius incertillus* Walk.; *Chilo simplex* Bult. (471)
Sugar cane	Sugar cane grub, *Lepidoderma albohirtum* (36, 37)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (403)
	Wireworms (Several species) (499)
	XIV. Vigor of Plant
Maize	European corn borer, *Pyrausta nubilalis* Hb. (71, 185, 366, 456, 536)
	Chinch bug, *Blissus leucopterus* (Say) (251, 404)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (487)
Potato	Potato flea beetles, *Epitrix cucumeris* Harris; *Epitrix suberinita* LeConte (211)
	XV. Adaptation to Soil and Other Conditions of Environment
Grape	*Phylloxera vitifoliae* Fitch (271)
Maize	Chinch bug, *Blissus leucopterus* (Say) (404, 406, 486)
	European corn borer, *Pyrausta nubilalis* Hb. (96, 261)
Rice	Leafhoppers, *Nephotettix bipunctatus* Fab.; *Nephotettix apicalis* Matsch. (369, 1)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (486, 487)
Tea	Tea mosquito bug, *Helopeltis theivora* Waterh. (6, 7)

different from breeding for various other plant characteristics which contribute to high yields of a quality product efficiently produced. Many plant characters may contribute toward resistance and many of these characters are interrelated with other desirable plant characters which may not necessarily contribute towards resistance. The fact that genetic segregation for all characters occurs simultaneously makes it highly undesirable to limit a breeding program to insect resistance alone. This fact applies also to a breeding program for any other plant characteristic short of the complete objective of high yields of a quality product efficiently produced. Breeding for insect resistance should, therefore, be included in a more generalized plant breeding program.

Variations in plants of significance in relation to insect attack have been reviewed in this paper, but such a review would not be complete without mention of the occurrence of variations within insect species which have been variously called biological, physiological or intra-specific races. Recent work has extended our knowledge of this subject to a great extent, and the close bearing that it has upon various problems of variation and evolution is becoming increasingly clear.

Variations within insect species probably was first suggested as long ago as 1864 by Walsh (540), an American worker who supposed from his observations on various wood-boring and plant-feeding beetles that races of this type attached to different food plants, must exist. Walsh's work appears to have attracted little attention at the time of publication, and was totally neglected until Craighead (105) called attention to it in 1923. This neglect is all the more surprising considering that the paper by Walsh appeared about the time of the publication of the "Origin of Species." It is remindful of the similar neglect of Mendel's classical paper which was published in 1865.

A biological race may be said to exist where the individuals of a species can be divided into groups, usually isolated to some extent by food preferences, occurring in the same locality and showing definite differences in biology; but with corresponding structural differences, either few and inconstant, or completely absent.

The presence of biological races in insect species and the probability of the development of new forms in the future produces some uncertainty concerning the period of time that present resistant varieties can remain free of insect damage. Certainly so many variations within the host plant and within insect species emphasizes the caution that must be exercised in expecting too much from the breeding of insect-resistant plants. The limited conditions of environment under which some plants are resistant and the complex factors that are responsible for resistance emphasize further the need for caution in a study of insect resistance.

Thorpe (513) has made a very excellent review of the literature dealing with biological races in insects. A total of 143 references is listed in his review. A few papers that were omitted by Thorpe, and most of those that have appeared since the publication of his review in 1930 are shown in the bibliography here. The following

references are included: Börner (53), Davidson (133), Ebeling (156), Harland and Atteck (217), Haviland (230), Lal (314), Moore (376), Stellwaag (497), Topi (514, 515, 516), and Walsh (540).

Records of plant breeding specifically for insect resistance involve several plant and insect species. Many of these records indicate that progress has been made, although the records are somewhat frag-

TABLE 3
RECORDS OF ACCOMPLISHMENTS IN BREEDING PLANTS FOR INSECT RESISTANCE

Host	Insect
Apple	Woolly apple aphid, *Eriosoma lanigerum* (Hausm.) (107, 284, 345, 494, 530)
Basket willow	Gall midge, *Rhabdophaga terminalis* H. Lw. (30)
Clover, Red	Potato leafhopper, *Empoasca fabae* (Harr.) (428)
Cotton	Black scale, *Saissetia nigra* Nietn. (214)
	Leaf blister mite, *Eriophyes gossypii* Banks (213, 215)
Gooseberry	Gooseberry witch-broom aphid, *Myzus houghtonensis* (139)
Grape	Grape Phylloxera, *Phylloxera vitifoliae* Fitch (48, 52, 54, 439)
Maize	Corn-leaf aphid, *Aphis maidis* Fitch (193)
	Corn-root aphid, *Aphis maidis-radicis* Forbes (193)
	European corn borer, *Pyrausta nubilalis* Hb. (192, 333, 334, 335, 336, 338, 363, 364, 365, 366)
	Chinch bug, *Blissus leucopterus* (Say) (250, 251, 404, 486)
	Corn ear worm, *Heliothis armigera* Hb. (95, 330, 430)
Oats	Frit fly, *Oscinella frit* L. (115)
Papaya (*Carica papaya*)	Papaya fruit fly, *Toxotrypana curvicauda* Gerst. (341)
Peach	Peach and prune root borer, *Synanthedon opalescens* H. Edw. (510)
Peas	Pea aphid, *Illinoia pisi* (Kalt.) (468)
Prune	Peach and prune root borer, *Synanthedon opalescens* H. Edw. (510)
	Aphids (species not indicated) (34)
Potato	Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (522)
Raspberry	Raspberry aphid, *Amphorophora rubi* Kalt. (258, 463, 464)
Sorghum	Chinch bug, *Blissus leucopterus* (Say) (124, 406, 408, 486, 487)
Strawberry	Strawberry aphid, *Capitophorus fragariae* (129, 491)
Sugar cane	*Lachnosterna smithii* Arrow (285)
	Sugar cane borer, *Diatraea saccharalis* F. (346)
Sugar beet	Sugar beet leafhopper, *Eutettix tenella* (Baker) (10, 74, 148, 161)
Sunflower	Sunflower moth, *Homoeosoma nebulella* Hb. (451)
Wheat	Hessian fly, *Phytophaga destructor* (Say) (72, 80, 387, 393, 400, 402, 403, 406)

mentary. In table 3 is compiled a list of references that have a bearing on breeding crop plants for insect resistance.

Bibliography

The preparation of a paper such as this is necessarily compilation, and the writer has made free use of the available literature. There is no doubt that some important papers dealing with the subject of host resistance have been missed, but an earnest attempt has been made to review and list all that could be located.

1. Abalos, R. Rice leafhoppers, *Nephotettix apicalis* Motch. and *Nephotettix bipunctatus* Fabr. Philippine Jour. Agr. **10**: 205–209. 1939.
2. Albrecht, H. R. Species and variety differences in resistance to aphid injury in vetch. Jour. Econ. Ent. **33**: 833–834. 1940.
3. Allen, Merle W., and Painter, Reginald H. Observations on the biology of the wheat stem maggot in Kansas. Jour. Agr. Res. **55**: 215–238. 1937.
4. Allen, T. C., and Rieman, G. H. Occurrence of hopperburn resistance and susceptibility in the potato. Amer. Pot. Jour. **16**: 139–142. 1939.
5. Anderson, Dean C., and Brown, Hubert M. Studies on Hessian fly infestation and some characters of the wheat culm. Jour. Amer. Soc. Agron. **28**: 479–483. 1936.
6. Andrews, E. A. A preliminary note on the present status of the mosquito-blight enquiry. Quart. Jour. Sci., Dept. Indian Tea Ass'n., Calcutta **4**: 119–129. 1919.
7. ———. Some notes on attempts to produce immunity from insect attack on tea. Proc. Ent. Soc., Pusa: 56–59. 1921.
8. ———. Factors affecting the control of the tea mosquito bug (*Helopeltis theivora* Waterh.). Indian Tea Ass'n., London **6**: 1–260. 1923. [Abstracted from Rev. Appl. Ent.]
9. Anonymous. Plant diseases and pests in Denmark in 1930. Tidsskr. Planteavl. **37**: 458–508. 1931. [Abstracted from Rev. Appl. Ent.]
10. ———. New sugar beet varieties for the curly-top area. U. S. Dept. Agr. Cir. 391. 1936.
11. Arant, F. S. Control of the cowpea curculio (*Chalcodermus aeneus* Boh.). Ala. Agr. Exp. Sta. Ann. Rpt. **43**: 1–22. 1932.
12. ———. Life history and control of the cowpea curculio. Ala. Agr. Exp. Sta. Bul. 246. 1938.
13. Atherton, Lee. Large, hard, borer-resistant bamboos have been propagated. Puerto Rico Agr. Exp. Sta. Rpt. **1937**. 26–37. 1937.
14. Back, E. A., and Pemberton, C. E. Susceptibility of citrus fruit to attack of Mediterranean fruit fly. Jour. Agr. Res. **3**: 311–330. 1915.
15. Baerg, W. J. I. The strawberry weevil. II. A false wireworm on strawberry. Ark. Agr. Exp. Sta. Bul. 185. 1923.
16. Bailey, Stanley F. The biology of the bean thrips. Hilgardia **7**: 467–522. 1933.
17. ———. The bean thrips. Calif. Agr. Exp. Sta. Bul. 609. 1937.
18. ———. Thrips of economic importance in California. Calif. Agr. Exp. Sta. Cir. 346. 1938.
19. ———. The factor of resistance to insect attack in plant breeding: vegetables. [Mss. for Jour. Econ. Ent. **34**: 1941.]
20. Bailey, W. K. Resistance of Puerto Rican wild lima beans to pod borers. Puerto Rico Exp. Sta. Rpt. 1938.
21. Baker, Howard. *Phylloxera devastatrix* Perg. on pecans. Jour. Econ. Ent. **28**: 681–685. 1935.
22. Ball, Carleton R., and Hastings, Stephen H. Grain sorghum pro-

duction in the San Antonio region of Texas. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 237. 1912.
23. BALL, E. D. The potato leafhopper and the hopperburn that it causes. Wis. Dept. Agr. Bul. 23: 76–102. 1919.
24. ———. Food plants of some Arizona grasshoppers. Jour. Econ. Ent. 29: 679–684. 1936.
25. BALLOU, H. A. The pink boll worm in Egypt 1916–1917. Min. Agr. Egypt, Cairo, pp. 106–108, 1920.
26. BARBER, G. W. The corn ear worm in southeastern Georgia. Ga. Agr Exp. Sta. Bul. 192. 1936.
27. ———. Preference of corn ear worm moths for sweet corn for oviposition. Jour. Econ. Ent. 39: 802–803. 1937.
28. BARBEY, A. La forêt française envisagée dans son ensemble au point de vue de la résistance aux attaques des insectes. [In French, German and English.] Comp. Rend. Un. Int. Inst. Rech. 1932 (Nancy), 721–727. 1933.
29. BARNES, H. F. On the resistance of basket willows to button gall formation. Ann. Appl. Biol. 17: 638–640. 1930.
30. ———. Further results of an investigation into the resistance of basket willows to button gall formation. Ann. Appl. Biol. 18: 75–82. 1931.
31. ———. On the gall midges injurious to the cultivation of willows; the bat willow gall midge, *Rhabdophaga terminalis* H. Lw. Ann. Appl. Biol. 19: 243–252. 1932.
32. ———. The biology of the chrysanthemum midge in England. Ann. Appl. Biol. 27: 71–91. 1940.
33. BATHELLIER, J. Dégats causés par les termites d'Indochine, aux végétaux vivants et aux bois d'oeuvre et les moyens d'y remédier. Trav Ve Congr. Int. Ent., Paris 1932. 2: 747–750. 1933. [Abstracted from Biol. Abst.]
34. BEACH, S. A., AND MANEY, T. J. Mendelian inheritance in *Prunus* hybrids. Amer. Breed. Ass'n. Ann. Rpt. 7: 214–226. 1912.
35. BECKER, G. C. Notes on the woolly aphis. Jour. Econ. Ent. 11: 245–255. 1918.
36. BELL, A. F. Report of the Division of Entomology and Pathology. Rpt. Bur. Sugar Exp. Stas., Queensland, Brisbane 34: 50–72. 1934.
37. ———. The resistance of the seedling S.J. 4 to grub damage in North Queensland. Cane Growers Quart. Bul., Queensland 2: 9–10. 1934.
38. ———. Report of the Division of Entomology and Pathology. Rpt. Bur. Sugar Exp. Stas., Queensland, Brisbane 36: 19–23. 1936.
39. ———. Report of the Division of Entomology and Pathology. Rpt. Bur. Sugar Exp. Stas., Queensland, Brisbane 37: 34–37. 1937.
40. BELYAEV, I. M., AND KHARCHENKO, V. V. Characters of cereals indicating their resistance against infestation by *Oscinosoma frit* L. [In Russian with English summary.] Plant Prot. 10: 50–60. 1936.
41. BENTON, CURTIS, AND FLINT, W. P. The comparative attractiveness of various small grains to the chinch bug. U. S. Dept. Agr. Cir. 508. 1938.
42. BEYER, A. H. The bean leafhopper and hopperburn with methods of control. Fla. Agr. Exp. Sta. Bul. 164: 61–88. 1922.
43. BIGGER, J. H. Resistance of certain wheat varieties to attack by the wheat stem maggot, *Meromyza americana* Fitch. Jour. Amer. Soc. Agron. 30: 448. 1938.
44. ———. Breeding corn for resistance to insect attack. [Mss. for Jour. Econ. Ent. 34: 1941.]
45. ———, FLINT, W. P., AND FARRAR, M. D. Fighting grasshoppers on Illinois farms. Ill. Agr. Exp. Sta. Cir. 466. 1937.
46. ———, HOLBERT, J. R., FLINT, W. P., AND LANG, A. L. Resistance

of certain corn hybrids to attack of southern corn rootworm. Jour. Econ. Ent. 31: 102–107. 1938.

47. Bioletti, Frederic T. The Phylloxera of the vine. Calif. Agr. Exp. Sta. Bul. 131. 1901.

48. ———, Flossfeder, F. C. H., and Way, A. E. Phylloxera resistant stocks. Calif. Agr. Exp. Sta. Bul. 331. 1921.

49. Blanchard, R. A., Bigger, John H., and Snelling, Ralph O. Resistance of corn strains to the corn ear worm, *Heliothis armigera* Hb. Jour. Amer. Soc. Agron. 33: 344–350. 1941.

50. ———, and Dudley, John E., Jr. Alfalfa plants resistant to the pea aphid. Jour. Econ. Ent. 27: 262–264. 1934.

51. Bonnet, L. O. Phylloxera resistant vineyards. Calif. Agr. Exp. Sta. Cir. 288. 1925.

52. Börner, C. Über reblaus-anfällige und-immune Reben. (On the susceptibility and immunity of vines to the attacks of the vine louse.) Biol. Centralblatt, Leipzig. 34 (1): 1–8. 1914. [Abstracted from Rev. Appl. Ent.]

53. ———. Zur Heimatfrage der Reblaus. (On the question of the original habitat of the vine louse.) Zeits. Ang. Ent. 8 (1): 163–167. 1921. [Abstracted from Rev. Appl. Ent.]

54. ———. (Contributions on breeding grape-vines resistant to *Phylloxera* and mildew. I. Introduction) Mitt Biol. Reichsanst. No. 49. 1934. [Abstracted from Rev. Appl. Ent.]

55. Bottger, G. T. Preliminary studies of the nutritive requirements of the European corn borer. Jour. Agr. Res. 60: 249–257. 1940.

56. Box, Harold E. La *Diatraea saccharalis* y las variedades da cana P.O.J.; Algunas observaciones y criticas. (*D. saccharalis* and the P.O.J. canes, some observations and criticisms.) Rev. Ind. Agr., Tucuman 18: 108–115. 1927–1928. [Abstracted from Rev. Appl. Ent.]

57. Boyce, A. M. Influence of host resistance and temperature during dormancy upon seasonal history of the walnut husk fly, *Rhagoletis completa* Cress. Jour. Econ. Ent. 26: 813–819. 1933.

58. ———. Bionomics of the walnut husk fly, *Rhagoletis completa.* Hilgardia 8: 363–579. 1934.

59. Breakey, E. P., Grunder, M. S., and Bauer, Karl. The resistance of plants to insect attack. West. Wash. Agr. Exp. Sta. Rpt. 1939. 1939.

60. Bremer, M. Importancia de los Pies inatacables por el Pulgon lanudo en las Plantaciones de Manzonos en la Argentina. (The importance of stocks resistant to the woolly aphis in the apple orchards of Argentina.) Anales Soc. Rur. Argentina, Buenos Aires 56 (15): 398–400. 1922. [Abstracted from Rev. Appl. Ent.]

61. Brues, C. T. The selection of food plants by insects, with special reference to lepidopterous larvae. Amer. Nat. 54: 313–332. 1920.

62. ———. Choice of food and numerical abundance among insects. Jour. Econ. Ent. 16: 46–51. 1923.

63. ———. The specificity of food plants in the evolution of *Phytophagus* insects. Amer. Nat. 58: 127–144. 1924.

64. Brunson, Arthur M., and Painter, Reginald H. Differential feeding of grasshoppers on corn and sorghums. Jour. Amer. Soc. Agron. 30: 334–346. 1938.

65. Bryson, Harry R. Observations on the summer activities of *Aphis maidis* Fitch. Jour. Econ. Ent. 27: 827–832. 1934.

66. Burgess, A. F. The gipsy moth and the brown-tail moth and their control. U. S. Dept. Agr. Farmers' Bul. 845. 1918.

67. Burk, Earl F., Cross, C. B., and Hixon, Ephriam. Variety test of sweet corn and its resistance to corn ear worm and smut injury.

68. Burlison, W. L., and Flint, W. P. Fight the chinch bug with crops. Ill. Agr. Exp. Sta. Cir. 268. 1923.
69. Buzacott, J. H. Rind hardness as an aid in beetle borer control. Queensland Soc. Sugar Cane Tech. Proc. 8th Ann. Conf. 143–152. 1937.
70. Caesar, L. The European corn borer in Ontario in 1936. Ent. Soc. Ontario 67th Ann. Rpt. 33–35. 1936.
71. Caffrey, D. J., and Worthley, L. H. The European corn borer, its present status and methods of control. U. S. Dept. Agr. Farmers' Bul. 1548. 1927.
72. Caldwell, R. M., and Compton, L. E. Wheat breeding for the combined resistance to disease and Hessian fly. Ind. Agr. Exp. Sta. Ann. Rpt. Dir. 1938. 39, 41–42.
73. Calhoun, P. W. Irregularity among cotton plants in time of fruiting as a factor affecting susceptibility to damage by the cotton weevil. Jour. Econ. Ent. **26**: 1125–1128. 1933.
74. Carsner, Eubanks. Curly-top resistance in sugar beets and tests of the resistant variety U. S. No. 1. U. S. Dept. Agr. Tech. Bul. 360. 1933.
75. Carter, Walter. Population of *Eutettix tenella* Baker and the osmotic concentration of its host plant. Ecology **8**: 350–352. 1927.
76. Carter, W. C. The toxic dose of mealy-bug wilt of pineapple. Phytopath. **27**: 971–981. 1937.
77. Cartwright, O. L. The rice weevil and associated insects in relation to shuck lengths and corn varieties. S. C. Agr. Exp. Sta. Bul. 266. 1930.
78. ———. Rice weevil in corn. S. C. Agr. Exp. Sta. 49th Ann. Rpt. 1935–36. 44–45. 1937.
79. ———. Rice weevil in corn. S. C. Agr. Exp. Sta. 50th Ann. Rpt. 1936–37. 52–54. 1938.
80. Cartwright, W. B., and Wiebe, G. A. Inheritance of resistance to the Hessian fly in the wheat crosses Dawson × Poso and Dawson × Big Club. Jour. Agr. Res. **52**: 691–695. 1936.
81. Chapman, A. J. Pink boll worm, varietal resistance. Texas Agr. Exp. Sta. Ann. Rpt. 1937. 62–63. 1937.
82. Chapman, Isaac. Some observations on the Hessian fly; written in the year 1797. Mem. Phila. Soc. Prom. Agr. **5**: 143–153. 1826.
83. Chapman, Paul J. Viability of eggs and larvae of the apple maggot, *Rhagoletis pomonella* Walsh, at 32° F. N. Y. Agr. Exp. Sta. (Geneva) Tech. Bul. 206. 1933.
84. Chesnokov, P. G. Relationship between distribution of various wheat forms and their resistance to attacks of frit-fly, *Oscinella frit* L. Comp. Rend. Acad. Sci. **22**: 282–286. Moscow, 1939. [In English.]
85. ———. Geographic variation of wheats in their resistance to Hessian fly, *Phytophaga destructor* Say. Comp. Rend. Acad. Sci. **23**: 287–291. Moscow, 1939. [In English.]
86. ———. Spring wheats resistant to Hessian fly. Selektzia; Semenovodstvo (Plant Breeding and Seed Growing) **10**: 12–13. 1939.
87. Chester, Kenneth S. The problem of acquired physiological immunity in plants. Quart. Rev. Biol. **8**: 129–154. 1933.
88. Chopra, R. L. On the structure, life history, economic importance and distribution of the cocks-foot moth, *Glyphipteryx fischeriella,* Zell. Ann. Appl. Biol. **12**: 359–397. 1925.
89. Chowdhury, K. A. The liability of some Indian timbers to *Lyctus* attack. Indian Forester **59**: 164–170. 1933.
90. Clarke, S. H. Vessel size and the liability of woods to *Lyctus* attack. Gr. Brit. For. Prod. Res. Bul. 2. 1928.
91. ———. On the relationship between vessel size and *Lyctus* attack

92. CLEARE, L. D. Moth borer damage in relation to sugar cane varieties in British Guiana. Trop. Agr. 9: 264–271. 1932.
93. ————. Sugar cane moth borer investigations in British Guiana; The present position. Agr. Jour. Brit. Guiana 5: 13–21. 1934.
94. COLE, C. F. Two new varieties of blight-proof apple. Jour. Dept. Agr., Victoria 20: 491–495. 1922.
95. COLLINS, G. N., AND KEMPTON, J. H. Breeding sweet corn resistant to the corn ear worm. Jour. Agr. Res. 11: 549–572. 1917.
96. CONREY, G. W. Soil type influences accumulation of European corn borer. Ohio Agr. Exp. Sta. Bul. 431. 1929.
97. COOK, O. F. Weevil resisting adaptations of the cotton plant. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 88. 1906.
98. ————. Relation of drought to weevil resistance in cotton. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 220. 1911.
99. ————. Cotton improvement under weevil conditions. U. S. Dept. Agr. Farmers' Bul. 501. 1912.
100. COOPER, T. P. Wireworms and tobacco. Ky. Agr. Exp. Sta. Ann. Rpt. Dir. 48th, p. 26, 1936; 49th, p. 33, 1937; 50th, p. 45, 1938; 51st, p. 16, 1939.
101. COPPER, R. R., DUNGAN, G. H., LANG, A. L., BIGGER, J. H., KOEHLER, BENJAMIN, AND BOLIN, OREN. Illinois corn performance tests 1939. Ill. Agr. Exp. Sta. Bul. 463. 1940.
102. CORBETT, G.H. Some preliminary observations on the coffee berry beetle borer, *Stephanoderes* (*Cryphalus*) *hampei* Ferr. Malayan Agr. Jour. 21: 8–22. 1933.
103. COWLAND, J. W. Gezira Entomological Section, G. A. R. S. final report on experimental work, 1932–3. Gezira Agr. Res. Serv. Ann. Rpt. (Sudan Govt.) 1933. pp. 107–125. 1934. [Abstracted from Rev. Appl. Ent.]
104. CRAIGHEAD, F. C. Hopkins host selection principle as related to certain cerambycid beetles. Jour. Agr. Res. 22: 189–220. 1921.
105. ————. The host selection principle as advanced by Walsh. Canad. Ent. 76–79. 1923.
106. ————. Relation between mortality of trees attacked by the spruce budworm, *Cacoecia fumiferana* Clem. and previous growth. Jour. Agr. Res. 20: 541–555. 1925.
107. CRANE, M. B. Breeding immune rootstocks. Ann. Appl. Biol. 24: 188–195. 1937.
108. ————, GREENSLADE, R. M., MASSEE, A. M., AND TYDEMAN, H. M. Studies on the resistance and immunity of apples to the woolly aphis, *Eriosoma lanigerum* (Hausm.). Jour. Pomol. & Hort. Sci. 14: 137–163. 1936.
109. CRESSMAN, A. W. Biology and control of *Chrysomphalus dictyospermi* (Morg.) Jour. Econ. Ent. 26: 696–706. 1933.
110. CUMMINS, J. E., AND WILSON, H. B. The pore size (vessel diameter) of some Australian timbers and their susceptibility to attack by the powder post borer, *Lyctus brunneus* Stephens. Jour. Council Sci. & Ind. Res., Australia 7: 225–233. 1934.
111. ————, AND ————. The starch content of some Australian hardwoods in relation to their susceptibility to attack by the powder post borer, *Lyctus brunneus* Stephens. Jour. Council Sci. & Ind. Res., Australia 8: 101–110. 1935.
112. CUNLIFFE, NORMAN. Studies on *Oscinella frit* L. Ann. Appl. Biol. 12: 276–286. 1925.
113. ————. Studies on *Oscinella frit* L. Ann. Appl. Biol. 16: 135–170. 1929.
114. ————. Studies on *Oscinella frit* L. Ann. Appl. Biol. 17: 549–553. 1930.

115. ————. Studies on *Oscinella frit* L. Inheritance of resistance of oats to attack by the fly and the combination of resistance with other characters of agricultural importance. Ann. Appl. Biol. **23**: 822–844. 1936.

116. ————, AND FRYER, J. C. F. *Oscinella frit* L. An investigation to determine how far varietal differences may influence infestation of the oat plant. Ann. Appl. Biol. **11**: 465–481. 1924.

117. ————, AND ————. Studies on *Oscinella frit* L. Ann. Appl. Biol. **12**: 508–515. 1925.

118. ————, ————, AND GIBSON, GORDON W. Studies on *Oscinella frit,* L. The correlation between stage of growth of stem and susceptibility to infestation. Ann. Appl. Biol. **16**: 516–526. 1929.

119. CUTRIGHT, C. R., AND MORRISON, H. E. Varietal susceptibility to codling moth injury. Jour. Econ. Ent. **28**: 107–109. 1935.

120. DAHMS, R. G. Field cages allow accurate test of crop's resistance. Phosphorus in soil increases sorghum's resistance to chinch bugs. Okla. Agr. Exp. Sta. Bien. Rpt. 1937–38. pp. 84–86, 1939.

121. ————, AND FENTON, F. A. Plant breeding and selecting for insect resistance. Jour. Econ. Ent. **32**: 131–134. 1939.

122. ————, AND ————. The effect of fertilizers on chinch bug resistance in sorghums. Jour. Econ. Ent. **33**: 688–692. 1940.

123. ————, AND PAINTER, REGINALD H. Rate of reproduction of the pea aphid on different alfalfa plants. Jour. Econ. Ent. **33**: 482–485. 1940.

124. ————, AND MARTIN, J. H. Resistance of F_1 sorghum hybrids to the chinch bug. Jour. Amer. Soc. Agron. **32**: 141–147. 1940.

125. ————, AND SNELLING, RALPH O. Factors influencing resistance of sorghums to chinch bugs discovered. Okla. Agr. Exp. Sta. Bien. Rpt. 1935–36. pp. 34–38, 1936.

126. ————, SNELLING, RALPH O., AND FENTON, F. A. Effect of different varieties of sorghum on biology of the chinch bug. Jour. Amer. Soc. Agron. **28**: 160–161. 1936.

127. ————, ————, AND ————. Effect of several varieties of sorghum and other host plants on biology of the chinch bug. Jour. Econ. Ent. **29**: 1147–1153. 1936.

128. DANIELS, LESLIE B. Controlling Colorado potato pests. Colo. Agr. Exp. Sta. Bul. 437. 1937.

129. DARROW, GEORGE M., WALDO, GEORGE F., AND SCHUSTER, C. E. Twelve years of strawberry breeding. Jour. Hered. **24**: 391–402. 1933.

130. DAVIDSON, J. Biological studies of *Aphis rumicis* L. reproduction on varieties of *Vicia faba*. Ann. Appl. Biol. **9**: 135–145. 1922.

131. ————. Biological studies of *Aphis rumicis* L. The penetration of plant tissues and the source of food supply of aphids. Ann. Appl. Biol. **10**: 35–54. 1923.

132. ————. Biological studies of *Aphis rumicis* L. Factors affecting the infestation of *Vicia faba* with *Aphis rumicis*. Ann. Appl. Biol. **12**: 472–507. 1925.

133. ————. The biological and ecological aspects of migration in aphids. Sci. Prog. **22** (85): 57–69. 1927.

134. DAVIDSON, W. M., AND MONGARET, R. L. The grape Phylloxera in California. U. S. Dept. Agr. Bul. 903. 1921.

135. DAVIS, J. J. The control of three important wheat pests in Indiana. Purdue Agr. Exp. Sta. Cir. 82. 1918.

136. ————. The European corn borer: past, present and future. Jour. Econ. Ent. **28**: 324–333. 1935.

137. DEAN, G. A., AND SMITH, R. C. Insects injurious to alfalfa in Kansas. Kans. State Bd. Agr. 29th Bien. Rpt. 202–249. 1935.

138. DELONG, D. M. Insecticidally induced immunity in plants to sucking insects. Science **80** (2075): 320–321. 1934.

139. ———, AND JONES, MERLIN P. Control measures for the houghton gooseberry aphis, with special reference to plant resistance. Jour. Econ. Ent. **19**: 40–43. 1926.
140. DEVEREAUX, W. L. The Hessian fly's favorite wheat. Rural New Yorker **37**: 376–377. 1878.
141. DEVERTEUIL, L. L. Sugar cane varieties in Antigua in relation to (a) infestation, (b) field losses by the moth borer, *Diatraea saccharalis* Fabr. Trop. Agr. **13** (5): 130–135. 1936.
142. DHARMARAJULU, K. The nature of resistance in cotton plants to stem weevil. Assoc. Econ. Biol. (Coimbatore) Proc. 21–31. 1935.
143. DIETER, C. E., AND WILSON, H. F. Injury to pea vines caused by the feeding of the pea aphid. Jour. Agr. Res. **59**: 805–814. 1939.
144. DIRKS, C. O. Larval production and adult emergence of the apple fruit fly in relation to apple varieties. Jour. Econ. Ent. **28**: 198–203. 1935.
145. DITMAN, L. P., AND CORY, E. N. Corn earworm studies. Md. Agr. Exp. Sta. Bul. 348: 525–543. 1933.
146. ———, AND ———. The corn earworm. Md. Agr. Exp. Sta. Bul. 399. 1936.
147. DOHANIAN, S. M. *Melissopus latiferreanus* (Walsi.) as a pest of filberts in the northwest. Jour. Econ. Ent. **33**: 852–856. 1940.
148. DOUGLASS, J. R., WAKELAND, CLAUDE, AND GILLETT, JOSEPH A. Field experiments for control of the beet leafhoppers in Idaho, 1936–37. Jour. Econ. Ent. **32**: 69–78. 1939.
149. DUDLEY, J. E., AND WILSON, H. F. Combat potato leafhopper with Bordeaux. Wis. Agr. Exp. Sta. Bul. 334. 1921.
150. DUNGAN, G. H., LANG, A. L., BIGGER, J. H., KOEHLER, BENJAMIN, AND BOLIN, OREN. Illinois corn performance test, 1938. Ill. Agr. Exp. Sta. Bul. 450. 1939.
151. ———, SNELLING, RALPH O., MUMM, W. J., BIGGER, J. H., AND LANG, A. L. Illinois corn performance test, 1937. Ill. Agr. Exp. Sta. Bul. 440. 1938.
152. DUNHAM, R. S. Stem maggot injury among wheat varieties. Jour. Amer. Soc. Agron. **26**: 978–980. 1934.
153. DUNNAM, E. W. Cotton boll growth in relation to boll weevil injury. Jour. Econ. Ent. **19**: 589–593. 1926.
154. ———, AND CLARK, J. C. Thrip damage to cotton. Jour. Econ. Ent. **30**: 855–857. 1937.
155. ———, AND ———. The cotton aphid in relation to the pilosity of cotton leaves. Jour. Econ. Ent. **31**: 663–666. 1938.
156. EBELING, WALTER. Host-determined morphological variations in *Lecanium corni*. Hilgardia **11**: 613–631. 1938.
157. EDGERTON, C. W. A study of wilt resistance in the seed bed. Phytopath. **8**: 5–14. 1918.
158. EICHMANN, R. D., AND WEBSTER, R. L. The influence of alfalfa on the abundance of the pea aphid on peas grown for canning in southeastern Washington. Wash. Agr. Exp. Sta. Bul. 389. 1940.
159. ELLINGER, T., AND CHORINE, V. Report on a corn variety test under corn borer conditions. Int. Corn Borer Invest. Sci. Rpt., Int. Live Stock Exp. Chicago, Ill., **3**: 99–100. 1930.
160. ———, AND ———. Comparaison de la résistance du Mais Sud- African et du Mais Américain. Á l'infection par le Pyrale du Mais. Ann. Inst. Pasteur **46**: 480–481. 1931.
161. ESAU, KATHERINE. Studies of the breeding of sugar beets for resistance to curly top. Hilgardia **4**: 415–440. 1930.
162. EVANS, A. C. Physiological relationships between insects and their host plants. I. The effect of the chemical composition of the plant on reproduction and production of winged forms in *Brevicoryne brassicae* L. (Aphididae). Ann. Appl. Biol. **25**: 558–572. 1938.
163. FAIRCHILD, DAVID. The world was my garden. p. 404. 1938.

164. FARLEMAN, M. G. Observations on the habits of flies belonging to the genus *Rhagoletis*. Jour. Econ. Ent. **26**: 825–828. 1933.
165. FARRAR, M. D. Control of borers in newly set shade trees. Jour. Econ. Ent. **32**: 634–638. 1939.
166. FELT, E. P. Recent experiments with the codling moth. Jour. Econ. Ent. **3**: 474–477. 1910.
167. ———, AND BROMLEY, S. W. Developing resistance or tolerance to insect attack. Jour. Econ. Ent. **24**: 437–443. 1931.
168. FENNAH, R. G. A summary of experimental work on varietal resistance of sugar cane to *Tomaspis saccharina*, 1936–1939. Trop. Agr. **16**: 233–240. 1939.
169. FENTON, F. A., AND HARTZELL, A. Bionomics and control of the potato leafhopper. Iowa Agr. Exp. Sta. Res. Bul. 78. 1923.
170. FEYTAUD, J. La Question Phylloxerique. La Crise des Porte-Greffes en Espagne. (The Phylloxera question. The crisis of grafted vines in Spain.) Bul. Soc. Vulg. Zool. Agric. (Bordeaux) **19**: 97–100, 113–116. 1920. [Abstracted from Rev. Appl. Ent.]
171. FICHT, G. A. Some observations in the planting date of corn and its relation to European corn borer populations. Jour. Econ. Ent. **24**: 380–386. 1931.
172. ———. Relative resistance of selected strains of corn to European corn borer. Jour. Econ. Ent. **29**: 687–691. 1936.
173. FIFE, J. M., AND FRAMPTON, V. L. The pH gradient extending from the phloem into the parenchyma of the sugar beet and its relation to the feeding behavior of *Eutettrix tenellus*. Jour. Agr. Res. **53**: 581–593. 1936.
174. FITCH, ASA. The Hessian fly. Amer. Quart. Jour. Agr. & Sci. **4**: 244–267. 1847; **5**: 1–27. 1847.
175. FLEMING, W. E., AND METZGER, F. W. Control of the Japanese beetle on fruit and shade trees. U. S. Dept. Agr. Cir. 237. 1936.
176. FLETCHER, T. B. Report of the Imperial Entomologist. Sci. Rpts. Agr. Res. Inst. Pusa & Calcutta, pp. 86–103, 1918 and 1919.
177. ———. Annotated list of Indian crop pests. Third Ent. Meeting Proc. Pusa and Calcutta, pp. 33–314, 1919 and 1920.
178. FLINT, W. P. The chinch bug. Jour. Econ. Ent. **28**: 333–341. 1935.
179. ———. Chinch bug resistance shown by certain varieties of corn. Jour. Econ. Ent. **14**: 83–85. 1921.
180. ———, AND BIGGER, J. H. Biological control of insects through plant resistance. Can. Ent. **70**: 244–246. 1938.
181. ———, DUNGAN, G. H., AND BIGGER, J. H. Fighting the chinch bug on Illinois farms. Ill. Agr. Exp. Sta. Cir. 431. 1935.
182. ———, HAYES, W. P., DUNGAN, G. H., AND YOUNG, A. L. Drouth checks corn borer advance in 1930. Ill. Agr. Exp. Sta. Cir. 367. 1931.
183. ———, ———, ———, AND ———. No corn borer found in Illinois in 1931. Ill. Agr. Exp. Sta. Cir. 384. 1932.
184. ———, AND HACKLEMAN, J. C. Corn varieties for chinch bug infested area. Ill. Agr. Exp. Sta. Bul. 243. 1923.
185. ———, ———, BAUER, F. C., AND BLAUSER, I. P. Learning to live with the European corn borer. Ill. Agr. Exp. Sta. Cir. 321. 1928.
186. FORBES, S. A. The corn leaf-louse, *Aphis maidis* Fitch. *In* State Ent. Ill. Rpt. 23. 123–133. 1905.
187. ———. Aspects of progress in economic entomology. Jour. Econ. Ent. **2**: 25–35. 1909.
188. FOSTER, W. R., AND JEFFERY, C. E. Resistance of winter wheat to Hessian fly. Canad. Jour. Res. **15** (4): 135–140. 1937.
189. FRANCA, A. A proposito di una varieta di melo resistente alla tignola. (Regarding a variety of apple escaping severe infestation by the

apple moth.) Note Fruttic, Pistoia **11**: 50–52. 1933. [Abstracted from Rev. Appl. Ent.]
190. FRYER, J. C. F., AND COLLINS, J. E. Certain aspects of the damage to oats by the frit fly. Ann. Appl. Biol. **11**: 448–464. 1924.
191. GAINES, J. C. Factors influencing the activities of the cotton bollworm moth, *Heliothis obsoleta* Fab. Jour. Econ. Ent. **26**: 957–962. 1933.
192. GARDNER, V. R. Blends of certain corn strains prove resistant to European corn borer. Mich. Agr. Exp. Sta. Rpt. **1932**: 33–34. 1932.
193. GERNERT, W. B. Aphis immunity of Teosinte-corn hybrids. Science **46**: 390–392. 1917.
194. GILBERTSON, G. I. The wheat stem maggot. So. Dak. Agr. Exp. Sta. Bul. 217. 1925.
195. GILLETTE, C. P. A few injurious insects and their remedies. Iowa State Hort. Soc. Trans. **25**: 102–106. 1891.
196. GORDON, ALEXANDER. Locust damage on sugar cane. Sugar News (Manila) **16** (2): 81–82. 1935.
197. GOSSARD, H. A., AND HOUSER, J. S. The Hessian fly. Ohio Agr. Exp. Sta. Bul. 177. 1906.
198. GRAHAM, C. The plum curculio. Md. Agr. Exp. Sta. Bul. 419. 1938.
199. GRAHAM, S. A. Principles of forest entomology. 339 pp. 1929.
200. GRANOVSKY, A. A. Differentiation of symptoms and effect of leaf hopper feeding on histology of alfalfa leaves. Phytopath. **20**: 121. 1930.
201. GREENSLADE, R. M. The control of woolly aphis. Ann. Appl. Biol. **24**: 184–188. 1937.
202. ———, MASSEE, A. M., AND ROACH, W. A. A progress report on the causes of immunity to the apple woolly aphis, *Erisoma lanigerum*, Hausmann. East Malling (Kent) Res. Sta. Ann. Rpt. **21** (1933). pp. 220–224. 1934.
203. GREGER, H. G. Resistant cabbage. Market. Grow. Jour. **53**: 246. 1933.
204. GWYNN, A. M. Factors affecting the attacks of sucking insects on cotton. East Afr. Agr. Jour. **3**: 314–318. 1938.
205. ———. Report of the entomologist, Serere. Dept. Agr. Rpt. Uganda, 1937–38. pt. 2: 10–19. 1939.
206. HAAS, A. R. C. Factors in varietal susceptibility of walnut fruits to attack by the walnut-husk fly. Plant Phys. **12**: 721–736. 1937.
207. HAGEN, H. Monographie de Termiten. Linnaea Entomol. **10**: 1–144, 1855.
208. HALL, RALPH C. Cape Cod pitch pine: Its resistance to gipsy moth defoliation and its advantages as a forest tree. Jour. For. **33**: 169–172. 1935.
209. ———. Growth and yield of shipmast locust on Long Island and its relative resistance to locust borer injury. Jour. For. **35**: 721–727. 1937.
210. HAMILTON, CLYDE C. The biology and control of the chrysanthemum midge, *Diarthronomyia hypogaea* F. Low. Md. Agr. Exp. Sta. Bul. 269. 1924.
211. HANSON, ARTHUR J. The potato flea beetles, *Epitrix cucumeris* Harris and *Epitrix subcrinita* Leconte. Wash. Agr. Exp. Sta. Bul. 280. 1933.
212. HARGRAVES, H. Report of the government entomologist for 1934. (Cotton resistant to jassid.) Dept. Agr. Rpt. Uganda, 1934. pt. 2: 62–72. 1934.
213. HARLAND, S. C. Notes on resistance to cotton leaf-blister mite with special reference to budded cottons, and to cotton hybrids. West Indian Bul. **16**: 78–82. 1916.
214. ———. A note on resistance to black scale in cotton. West Indian Bul. **16**: 255–256. 1917.
215. ———. The inheritance of immunity to leaf-blister mite, *Eriophyes gossypii* Banks in cotton. West Indian Bul. **17**: 162–166. 1919.

216. ———. A suggested method for the control of certain bollworms in cotton. Empire Cotton Growers Rev. 6 (4): 333–334. 1929.
217. ———, and Atteck, O. M. Breeding experiments with biological races of *Trichogramma minutum* in the West Indies. Zeits. Ind. Abst. Vererb. 64 (1–2): 54–76. 1933. [Abstracted from Rev. Appl. Ent.]
218. Harman, S. W. The cranberry rootworm as an apple pest. N. Y. Agr. Exp. Sta. Bul. 692. 1940.
219. Harper-Gray, R. A. Frit fly on oats in the four northern counties. Jour. Min. Agr. 29: 1109–1114, 1923.
220. Harrington, C. D., and Searls, Ed. M. Influence of the physiological age of the pea plant on its recovery from aphid damage. Jour. Agr. Res. 60: 157–161. 1940.
221. ———, ———, and Brink, R. A. Devise a method of testing peas for aphid resistance. Wis. Agr. Exp. Sta. Ann. Rpt. (Part 2) Bul. 449. 64–67, 1940.
222. Harrison, J. W. H. Experiments on the egg laying instincts of the sawfly *Pontania salicis* Christ. and their bearing on the inheritance of acquired characters; with some remarks on a new principle in evolution. Royal Soc. London, Proc. B 101: 115–126. 1927.
223. Hart, P. C. De topboorderschade bij 2883 POJ. (Tip-borer injury in 2883 POJ cane.) Arch. Suikerind. Ned.-Ind. (1933), pp. 301–314, 1933. [Abstracted from Rev. Appl. Ent.]
224. Hartman, Glen. A study of potato psyllid yellows in Wyoming. Wyo Agr. Exp. Sta. Bul. 220. 1937.
225. Hase, Albrecht. Report on corn borer experiments 1927–1928. Int. Corn Borer Invest. Sci. Rpts., Int. Live Stock Exp., Chicago, 2: 77–84. 1929.
226. Haseman, L. An investigation of the supposed immunity of some varieties of wheat to the attack of Hessian fly. Jour. Econ. Ent. 9: 291–294. 1916.
227. ———, and McLane, S. R. What the station is doing for Missouri. Entomological investigations. Mo. Agr. Exp. Sta. Bul. 179. 1921.
228. Hatton, R. G. I. Introduction; II. The northern spy as a rootstock. Ann. Appl. Biol. 24: 169–180. 1937.
229. Havens, Jonathan N. Observations on the Hessian fly. Soc. Agr. New York Trans., Pt. 1: 89–107. 1792.
230. Haviland, Maud D. Bionomics of *Aphis grossulariae* Kalt. and *Aphis viburni* Schr. Proc. Cambridge Phil. Soc. 19: 266–269. 1919.
231. Hawthorn, L. R. Further studies of ear worm damage in sweet corn varieties in Texas. Proc. Amer. Soc. Hort. Sci. (1935) 33: 505–507. 1936.
232. ———, and Fletcher, R. K. Ear worm damage in sweet corn varieties. Proc. Amer. Soc. Hort. Sci. (1933) 30: 550–553. 1934.
233. Hayes, W. P. Observations on insects attacking sorghums. Jour. Econ. Ent. 15: 349–356. 1922.
234. ———. Biological races of insects and their bearing on host plant resistance. Ent. News 46 (1): 20–23. 1935.
235. ———, and Johnston, C. O. The reaction of certain grasses to chinch bug attack. Jour. Agr. Res. 31: 575–583. 1925.
236. Hazelhoff, E. H. Entomologisch onderzoek. (Entomological investigations.) Jaarsversl. Proefst. Java Suiker Ind. Surabaya (1929), pp. 113–134. 1930. [Abstracted from Rev. Appl. Ent.]
237. ———. Onderzoekingen omtrent den witten topboorder. (Investigations on the white-tip-borer of sugar cane.) Arch. Suikerind Ned.-Ind. 3: 361–363. 1931.
238. Headlee, Thomas J. Notes on the corn ear worm. Jour. Econ. Ent. 3: 149–157. 1910.

239. HEDRICK, U. P. The corn ear worm on Long Island. N. Y. Agr. Exp. Sta. 52nd Ann. Rpt. 1932–33, p. 70: 1934.
240. HEPPNER, MYER J. Breeding plants to resist insect and fungus attack. Better Crops 3 (5): 13–14. 1925.
241. HERR, E. A. The gladiolus thrips *Taeniothrips gladioli* M. & S. Ohio Agr. Exp. Sta. Bul. 537. 1934.
242. HINDS, W. E. Proliferation as a factor in the natural control of the Mexican boll weevil. U. S. Dept. Agr. Bur. Ent. Bul. 59. 1906.
243. ———. Reducing insect injury to seed corn. Ala. Agr. Exp. Sta. Bul. 176: 49–68. 1914.
244. HIXON, EPHRIAM. I. Weevil resistant cotton sought. II. Damage to corn by earworm influenced by tightness of husk. Okla. Agr. Exp. Sta. Bien. Rpt. 1935–36, 29–30. 1936.
245. ———. Variation in the susceptibility of brambles to the red-necked cane borer, *Agrilus ruficollis* (F.). Jour. Econ. Ent. **31**: 546. 1938.
246. ———. I. High yielding cotton varieties best where weevils are numerous. II. Cane borer checked by pruning and by planting resistant varieties. Okla. Agr. Exp. Sta. Bien. Rpt. 1937–38, 94–96. 1939.
247. HODGE, CHARLES IV. Growth and nutrition of *Melanoplus differentialis* Thomas (Orthoptera: Acrididae). I. Growth on a satisfactory mixed diet and on diets of single food plants. Phys. Zool. **6**: 306–328. 1933.
248. HODSON, W. E. H., AND JARY, S. G. A new insect pest of chrysanthemum. Jour. Min. Agr. **46**: 54–56. 1939.
249. HOERNER, J. L. Controlling the squash bug. Colo. Agr. Exp. Sta. Press Bul. 93. 1938.
250. HOLBERT, J. R., FLINT, W. P., AND BIGGER, J. H. Chinch bug resistance in corn—an inherited character. Jour. Econ. Ent. **27**: 121–124. 1934.
251. ———, ———, ———, AND DUNGAN, G. H. Resistance and susceptibility of corn strains to second brood chinch bugs. Iowa State Coll. Jour. Sci. **9**: 413–425. 1935.
252. HOLDAWAY, F. G. Differential susceptibility of varieties of garden bean to oviposition by melon fly, *Dacus cucurbitae* Coq. Haw. Ent. Soc. Proc. 1929 **10**: 421–422. 1940.
253. HOLLOWAY, T. E. Borer control by breeding sugar cane for resistance. Sugar Bul. **13** (11): 3–4, 6. 1935.
254. HOLLOWELL, E. A., MONTEITH, JOHN, AND FLINT, W. P. Leafhopper injury to clover. Phytopath. **17**: 399–404. 1927.
255. HORSFALL, J. L. The effect of feeding punctures of aphids on certain plant tissue. Pa. Agr. Exp. Sta. Bul. 182. 1923.
256. HOWARD, L. O. The twelve-spotted Diabrotica injuring fruit trees. Insect Life **1**: 58–60. 1889.
257. HSU, TIEN SIH. Resistance of sorghum to stem borers. Jour. Amer. Soc. Agron. **28**: 271–278. 1936.
258. HUBER, GLENN A., AND SCHWARTZE, C. D. Resistance in the red raspberry to the mosaic vector *Amphorophora rubi* Kalt. Jour. Agr. Res. **57**: 623–633. 1938.
259. HUBER, L. L. European corn borer. Ohio Agr. Ext. Serv. Bul. 8. 1926.
260. ———. European corn borer resistance. Ohio Agr. Exp. Sta. Bul. 579: 43–53. 1937.
261. ———. The European corn borer and corn hybrids. Ohio Agr. Exp. Sta. Bimon. Bul. **24** (197): 26–28. 1939.
262. ———, AND STRINGFIELD, G. H. Strain susceptibility to the European corn borer and the corn leaf aphid in maize. Science **92** (2382): 172. 1940.
263. HUME, A. N. An indication of the relative susceptibility of dent and

flint corn to injury by grasshoppers. Jour. Amer. Soc. Agron. **23**: 1071. 1931.

264. ————. Forage crop investigations. So. Dak. Agr. Exp. Sta. Ann. Rpt. (1933) p. 14. 1933.

265. HUNTER, H., AND LEAKE, H. M. Recent advances in agricultural plant breeding. 361 pp. 1933.

266. HUNTER, W. D. The boll weevil problem with special reference to means of reducing damage. U. S. Dept. Agr. Farmers' Bul. 344. 1909.

267. ————. The boll weevil problem with special reference to means of reducing damage. U. S. Dept. Agr. Farmers Bul. 512. 1912.

268. ————. The boll weevil problem with special reference to means of reducing damage. U. S. Dept. Agr. Farmers' Bul. 848. 1917.

269. ————, AND PIERCE, W. D. The Mexican cotton boll weevil. U. S. Dept. Agr. Ent. Bul. 114. 1912.

270. HUSAIN, M. A., AND LAL, K. B. Cotton jassids and hairiness of cotton plant. Indian Sci. Cong. Proc. (Hyderabad) **24**: 368. 1937.

271. HUSMANN, GEORGE C. Testing Phylloxera-resistant grape stocks in the vinifera regions of the United States. U. S. Dept. Agr. Tech. Bul. 146. 1930.

272. HUTCHINGS, C. B. The lilac leaf miner. Ontario Ent. Soc. Ann. Rpt. 55 (1924). 19–23. 1925.

273. HUTCHINSON, H. P., AND KEARNS, H. G. H. Insect pests of willow. Nature **125** (3145) : 201, 276. 1930.

274. IJIMA, K. Relation of oviposition of *Eucosma schistaceana* Snell. to the varieties of sugar cane. Jour. Jap. Assoc. Sugar Plant. Formosa **13** (9) reprint 9 pp., 1935. [Abstracted from Rev. Appl. Ent.]

275. IMMS, A. D. Recent advances in entomology. 374 pp. 1931.

276. ISAAC, P. V. Report of the second entomologist (Dipterist) in charge of scheme for research on insect pests of sugar cane. Sci. Rpt. Agr. Res. Inst. New Delhi, 1937–38. 129–131. 1939.

277. ISLEY, DWIGHT. The relation of leaf color and leaf size to boll weevil infestations. Jour. Econ. Ent. **21**: 553–559. 1928.

278. ————. Relationship between early varieties of cotton and boll weevil injury. Jour. Econ. Ent. **27**: 762–766. 1934.

279. ————. Relation of host to abundance of cotton bollworm. Ark. Agr. Exp. Sta. Bul. 320. 1935.

280. ————, AND ACKERMAN, A. J. Life history of codling moth in Arkansas with special reference to factors limiting abundance. Ark. Agr. Exp. Sta. Bul. 189. 1923.

281. JABLONOWSKI, JOZSEF. A Kukoricamoly (*Botys nubilalis* Hb.) Kiserletugyi Kozlemenyek, Budapest, **1**: 33–48, 1898. [Abstracted in U. S. Bur. Ent. & Pl. Quar., Mim.]

282. ————. Vedekezes a Kukoricamoly ellen. (Defense against the corn moth.) Moztelek, Budapest. **28** (16) : 626–628. 1918. [Abstract in U. S. Bur. Ent. & Pl. Quar., Mim.]

283. JANCKE, O. Ueber den Einfluss der Kalidüngung auf die Anfälligkeit der Apfelbaüme gegen Blutlaus, Blattlaus and Mehltau Zugleich II Mitteilung zur innertherapeutischen Schädlingsbekämpfung. (On the influence of potash manure on the susceptibility of apple trees to attack by the woolly aphis, leaf-aphis, and mildew.) Biol. Reichsanst. Landu Forstw. Arb. **20**: 291–302. 1933. [Abstracted from Rev. Appl. Ent.]

284. ————. (On the susceptibility to infestation by the woolly aphis of various apples, wild apples and their hybrids and on the breeding of immune edible apples and stocks.) Phytopath. Zeits. **10** (2) : 184–196, 1937. [Abstracted from Rev. Appl. Ent.]

285. JEPSON, W. F., AND EVANS, H. Un essai préliminaire sur la résistance des variétés de cannes à l'attaque du *Phytalus*. Rev. Agr. Maurice

81: 97–101, Port Louis, 1935. [Abstracted from Rev. Appl. Ent.]
286. Jewett, H. H. The resistance of certain red clovers and alfalfas to leafhopper injury. Ky. Agr. Exp. Sta. Res. Bul. 329. 1932.
287. ———. The resistance of leaves of red clover to puncturing. Jour. Econ. Ent. **26**: 1135–1137. 1933.
288. ———. The resistance of leaves of some pubescent red clovers to puncturing. Jour. Econ. Ent. **28**: 697–698. 1935.
289. ———. Wireworm injury to tobacco plants. Ky. Agr. Exp. Sta. Bul. 398. 1940.
290. Johnson, H. W., and Hollowell, E. A. Pubescent and glabrous characters of soybeans as related to resistance to injury by the potato leaf hopper. Jour. Agr. Res. **51**: 371–381. 1935.
291. Jones, Elmer T. Hordeum grasses as hosts of the Hessian fly. Jour. Econ. Ent. **29**: 704–710. 1936.
292. ———. Differential resistance to chinch bug attack in certain strains of wheat. Trans. Kans. Acad. Sci. **40**: 135–142. 1937.
293. ———. Infestation of grasses of the genus *Aegilops* by the Hessian fly. Jour. Econ. Ent. **31**: 333–337. 1938.
294. ———. Grasses of the tribe Hordeae as hosts of the Hessian fly. Jour. Econ. Ent. **32**: 505–510. 1939.
295. ———. A discussion of fly resistance in certain wheat varieties. Kans. Acad. Sci. Trans. **43**: 243–265. 1940.
296. Jones, H. A., Bailey, S. F., and Emsweller, S. L. Thrips resistance in the onion. Hilgardia **8**: 215–232. 1934.
297. ———, ———, and ———. Field studies of *Thrips tabaci* Lind. with special reference to resistance in onions. Jour. Econ. Ent. **28**: 678–680. 1935.
298. Keck, C. B. Relation of oviposition punctures of the Mediterranean fruit fly to the premature dropping of citrus fruits. Jour. Econ. Ent. **27**: 908–914. 1934.
299. Keen, F. P. Relative susceptibility of Ponderosa pines to bark-beetle attack. Jour. For. **34**: 919–927. 1936.
300. Kelsheimer, E. G., and Polivka, J. B. Correlation of corn borer survival with maturity of corn. Jour. Econ. Ent. **24**: 386–388. 1931.
301. Kemp, H. J. Studies of solid stem wheat varieties in relation to wheat stem sawfly control. Sci. Agr. **15**: 30–38. 1934.
302. Kennedy, P. B., and Mackie, W. W. Berseem, or Egyptian clover, *Trifolium alexandrinum*. Calif. Agr. Exp. Sta. Bul. 389. 1925.
303. ———, and Madson, B. A. The mat bean, *Phaseolus aconitifolius*. Calif. Agr. Exp. Sta. Bul. 396. 1935.
304. Kiesselbach, T. A., Anderson, Arthur, and Suneson, C. A. Winter wheat varieties in Nebraska. Nebr. Agr. Exp. Sta. Bul. 283. 1933.
305. Kirichenko, O. M. The resistance of the varieties of wheat to injuries by frit-fly (*Oscinella frit* L.) in the south Ukrainian Steppe. (In Ukrainian with English summary) Plant Breed. Genet. Inst. Proc. (Odessa) **1**: 34–77. 1935.
306. Kleine, R. Die Anfälligkeit bzw. Widerstandsfähigkeit einzelner Hafersorten gegen den Befall durch *Oscinis frit* L. (The susceptibility or resistance of particular varieties of oats to attack by *Oscinella frit*.) Zeit. Schädlingsbekämpfung **1**: 2–12. 1923. [Abstracted from Rev. Appl. Ent.]
307. ———. Studien über die Widerstandsfähigkeit verschiedener Hafersorten gegen die Fritfliege. (Studies on the resistance of various varieties of oats to the frit fly.) Fortschr. Landw. **1**: 373–380. 1926. [Abstracted from Rev. Appl. Ent.]
308. ———. The immunity of oats from the attack of insect pests. Fortschr. Landw. **2**: 109–110. 1927. [Abstracted from Biol. Abst.]
309. Kobayashi, M., and Tamura, I. Differences in the degree of infestation by adult beetles of *Anomala rufocuprea* Motsch. of the varieties of soy bean. [In Japanese] Agr. & Hort. **14** (1): 37–40. 1939.

310. Konstantinova, M. Ya. Kohctahtnhoba (M.R.). (Experiments on the study of individual reaction to barley to injury by *Oscinella* (*Oscinosoma*) *frit* L.) [In Russian] Morbi Plantarum **15**: 125–136. 1926. [Abstracted from Rev. Appl. Ent.]
311. Körting, A. Über die Entwicklung der grauen Gerstenminierfliege *Hydrellia griseola* Fall. bei verschiedener Ernährung. Zeits. Pflanzenkr. **41**: 321–333. 1931.
312. Kozlovsky, S. Le cycle biologique de *Leptinotarsa decemlineata* Say en différentes variétés de tomate, *Solanum lycopersicum* L. Rev. Zool. Agr. **35** (7): 105–108. 1936.
313. Kyle, C. H. Shuck protection for ear corn. U. S. Dept. Agr. Bul. 708. 1918.
314. Lal, K. B. Biological races in *Psyllia mali* Schmidberger. Nature **132**: 834. 1933.
315. ————. Anti-jassid resistance in the cotton plant. Curr. Sci. (Bangalore) **6** (3): 88–89. 1937.
316. Leach, Julian Gilbert. Insect transmission of plant diseases. 615 pp. 1940.
317. Lees, A. H. "Reversion" and resistance to big bud in black currants. Ann. Appl. Biol. **5**: 11–27. 1918.
318. ————. Insect attack and the internal condition of the plant. Ann. Appl. Biol. **13**: 506–515. 1926.
319. LePelley, R. Studies on the resistance of apples to the woolly aphis, *Eriosoma lanigerum* Hausm. Jour. Pomol. & Hort. Sci. **6**: 209–241. 1927.
320. Lindblom, A., and Mühlow, J. Tva undersökningar rörande vetemyggans skadegörelse och ekonomiska betydelse. (Two investigations on the harmfulness and economic importance of wheat midges.) Medd. Cent. Anst. Försoksv. Jordbr. No. 420. 24 pp. 1932. [Abstracted from Rev. Appl. Ent.]
321. Lindley, George. A guide to the orchard and kitchen garden. 597 pp. 1831.
322. Lochhead, W. A few notes on the ecology of insects. Ent. Soc. Ontario, Ann. Rpt. **48**: 85–91. 1918.
323. MacLeod, G. F. Some examples of varietal resistance of plants to insect attack. Jour. Econ. Ent. **26**: 62–67. 1933.
324. ————. Plant varietal resistance to insect injury. Cornell Univ. Agr. Exp. Sta. 48th Ann. Rpt. 1935.
325. ————, and Maxwell, Kenneth E. Experiments to control hairy chinch bug infestating turf on Long Island. Jour. Econ. Ent. **30**: 432–437. 1937.
326. Mahoney, C. H. What the plant breeder can and should do for the vegetable grower. Breeding for insect resistance. Ohio Veg. Grow. Ass'n Proc. **19**: 57–59. 1934.
327. Mail, G. Allen. Food preferences of grasshoppers. Jour. Econ. Ent. **24**: 767–768. 1931.
328. Malenotti, E. Una varieta di melo resistente alla tignola. (A variety of apple resistant to *Hyponomeuta padellus*.) Atti Accad. Agric. Sic. Lett. **11** (5): 1933. [Abstracted from Rev. Appl. Ent.]
329. Maltais, J. B. Resistance of some varieties of peas to the pea aphid, *Illinoia pisi* Kalt. Ontario Ent. Soc. 67th Ann. Rpt. 40–45. 1936.
330. Mangelsdorf, P. C. Growing sweet corn in Texas. Tex. Agr. Exp. Sta. Cir. 69. 1934.
331. Marchal, P., Trouvelot, B.. Dixmeras, (—), and Grison (—). Variabilité de l'attaque du doryphore sur diverses solanées tubérifères. Comp. Rend. Acad. Agr. Fr., Paris **21** (31): 1169–1175. 1935. [Abstracted from Rev. Appl. Ent.]
332. Marlowe, R. H. Susceptibility of two tomato varieties to infestations by *Chaetodacus cucurbitae* (Coq.). Haw. Ent. Soc. Proc. **9**: 407–408. 1937.

333. MARSTON, A. R. Breeding corn for resistance to the European corn borer. Jour. Amer. Soc. Agron. **22**: 986–992. 1930.
334. ————. Breeding European corn borer resistant corn. Jour. Amer. Soc. Agron. **23**: 960–964. 1931.
335. ————. Recent progress in breeding borer resistant corn. Mich. Agr. Exp. Sta. Quart. Bul. **15** (4): 264–268. 1933.
336. ————. Michigan hybrid No. 561 a borer resistant field corn. Mich. Agr. Exp. Sta. Quart. Bul. **18** (4): 223–225. 1936.
337. ————, AND DIBBLE, C. B. Investigations of corn borer control at Monroe, Michigan. Mich. Agr. Exp. Sta. Spec. Bul. 304. 1930.
338. ————, AND MAHONEY, C. H. Progress report on breeding sweet corn for corn borer resistance. Proc. Amer. Soc. Hort. Sci. **29**: 472–476. 1932.
339. MARTIN, HUBERT. The scientific principle of plant protection. 20–29. 1928.
340. MASAITIS, A. I. On the study of *Elaterids* in Siberia. Izv. Sibirsk Kraev. Stantz. Zashch. Rast., Tomsk **2** (5): 53–65. 1927. [Abstracted from Rev. Appl. Ent.]
341. MASON, ARTHUR C. Biology of the papaya fruit fly, *Toxotrypana curvicauda,* in Florida. U. S. Dept. Agr. Bul. 1081. 1922.
342. ————. The economic importance of the Mediterranean fruit fly to Hawaiian horticulture. Hawaiian Ent. Soc. Proc. 1931 **8**: 163–178. 1932.
343. MASON, T. G., AND JONES, G. H. A first survey of factors inhibiting the development of the cotton plant in southern Nigeria. Nigeria Dept. Agr. Ann. Bul. 3. 11–30. 1924. [Abstracted from Rev. Appl. Ent.]
344. ————, AND PHILLIS, E. A note on a new method of control for insect pests of the cotton plant. Empire Cotton Grow. Rev. **14**: 308–309. 1937.
345 MASSEE, A. M. The resistance of certain apple stocks to attacks of the woolly aphis. East Malling (Kent) Res. Sta. Ann. Rpt. Supp. II. (1928–1930). 202–205. 1931.
346. MATHES, R., INGRAM, J. W., AND HALEY, W. E. Preliminary report on studies of progenies of sugar cane crosses for susceptibility to sugar cane borer, *Diatraea saccharalis* F. injury in Louisiana. Int. Soc. Sug. Cane Tech. Proc. **6**: 581–589. 1939.
347. MAUGHAN, FRANK B. Varietal differences in insect populations and injuries to potatoes. Amer. Potato Jour. **14**: 157–161. 1937.
348. ————, AND MACLEOD, G. F. Further studies on onion varieties and onion thrips. Jour. Econ. Ent. **29**: 335–339. 1936.
349. MCCOLLOCH, J. W. A study of the oviposition of the corn ear worm with relataion to certain phases of the life economy and measures of control. Jour. Econ. Ent. **13**: 242–255. 1920.
350. ————. The corn leaf aphis, *Aphis maidis* Fitch in Kansas. Jour. Econ. Ent. **14**: 89–94. 1921.
351. ————. The Hessian fly in Kansas. Kans. Agr. Exp. Sta. Tech Bul. 11. 1923.
352. ————. The resistance of plants to insect injury. Kans. State Hort. Soc. Bien. Rpt. **37**: 196–208. 1924.
353. ————, AND SALMON, S. C. Relation of kinds and varieties of grain to Hessian fly injury. Jour. Agr. Res. **12**: 519–527. 1918.
354. ————, AND ————. The resistance of wheat to the Hessian fly—a progress report. Jour. Econ. Ent. **16**: 293–298. 1923.
355. MCDOUGALL, W. A. The wireworm pest *Lacon variabilis* Cand. and its control in central Queensland's sugar cane fields. Agr. Jour. Queensland. **42**: 690–726. 1934.
356. ————. The wireworm pest, *Lacon variabilis* Cand. and its control in central Queensland's sugar cane fields. Sugar Exp. Sta., Brisbane. Ent. Bul. 22. 1935.

357. McINDOO, N. E. The relative attractiveness of certain solanaceous plants to the Colorado potato beetle, *Leptinotarsa decemlineata* Say. Proc. Ent. Soc. Wash. **37**: 36–42. 1935.
358. McKINNEY, K. B. Physical characteristics on the foliage of beans and tomatoes that tend to control small insect pests. Jour. Econ. Ent. **31**: 630–631. 1938.
359. MELIS, ANTONIO. Una grave infestazione di *Dendrolimus pini* L. nell'Isola di Lussino. **25**: 293–295. 1938. [Abstracted from Biol. Abst.]
360. METCALFE, MARGOT E. *Dasyneura leguminicola* (Lint.) the clover seed midge. Ann. Appl. Biol. **20**: 185–204. 1933.
361. METZGER, F. W., AND GRANT, D. H. Repellency to the Japanese beetle of extracts made from plants immune to attack. U. S. Dept. Agr. Tech. Bul. 299. 1932.
362. ———, VAN DER MEULEN, P. A., AND MELL, C. W. The relation of the sugar content and odor of clarified extracts of plants to their susceptibility to attack by the Japanese beetle. Jour. Agr. Res. **49**: 1001–1008. 1934.
363. MEYERS, M. T. Breeding varieties of corn to meet the Europeon corn borer. Ohio Agr. Exp. Sta. Ann. Rpt. 47. Bul. 431: 33–34. 1929.
364. ———. Breeding varieties of corn resistant to the European corn borer. Ohio Agr. Exp. Sta. Ann. Rpt. 48. Bul. 446: 32–34. 1930.
365. ———, CUTLER, J. S., AND NEISWANDER, C. R. Corn breeding in relation to the corn borer problem. *In* The European corn borer and its environment, by L. L. Huber, C. R. Neiswander, and R. M. Salter. Ohio Agr. Exp. Sta. Bul. 429: 143–147. 1928.
366. ———, HUBER, L. L., NEISWANDER, C. R., RICHEY, F. D., AND STRINGFIELD. G. H. Experiments on breeding corn resistant to the European corn borer. U. S. Dept. Agr. Tech. Bul. 583. 1937.
367. MILES, H. W., AND COHEN, M. Entomological field station, Warburton, Cheshire. Report for the year 1938. Jour. Lancs. Agr. Soc. 1939 Rpt. 1939.
368. MILLIKEN, F. B. Methods of controlling grasshoppers. Kans. Agr. Exp. Sta. Bul. 215. 1916.
369. MISRA, C. S. The rice leaf hoppers, *Nephotettix bipunctatus* Fabr., and *Nephotettix apicalis* Matsch. Indian Dept. Agr. Mem. Ent. Ser. **5**: 207–237. 1920.
370. ———. The "American blight" or the woolly aphis (*Eriosoma lanigera* Haus.). Agr. Jour. India **15**: 627–635. 1920.
371. MIWA, Y., AND YANAGIHARA, M. The effect of *Elaterid* beetles on sugar cane planting in Formosa. Jour. Trop. Agr., Formosa **1** (3): 275–289. 1929.
372. MOERDYK, J. L. Cotton jassid in South Africa. Trop. Agr. **4**: 46–47. 1927.
373. MONTEITH, JOHN, JR., AND HOLLOWELL, E. A. Pathological symptoms in legumes caused by the potato leaf hopper. Jour. Agr. Res. **38**: 649–677. 1929.
374. MONZEN, K. The woolly apple aphis, *Eriosoma lanigera* Hausm. in Japan, with special reference to its life history and the susceptibility of the host plant. Int. Ent. Kongr. **3**: 249–275. Zürich. 1925. [Abstracted from Rev. Appl. Ent.]
375. ———. Host selection and immunity of plants. [In Japanese] Sci. Bul. Alumni Soc. Morioka (Japan) Coll. Agr. **11**: 29–34. 1936.
376. MOORE, WILLIAM. Studies of the resistant California red scale, *Aonidiella aurantii* Mask. in California. Jour. Econ. Ent. **26**: 1140–1161. 1933.
377. MORRILL, A. W., JR., AND LACROIX, D. S. Experiments on control of insects of tobacco, 1936. Conn. Agr. Exp. Sta. Bul. 391. 1937.
378. MOSHER, F. H. Food plants of the gipsy moth in America. U. S. Dept. Agr. Bul. 250. 1915.

379. MUHLOW, J. Studies and research on the wheat gall-midges, *C. tritici* and *Sitodiplosis mosellana,* and their control. I. Injury caused by the larvae of *C. tritici* in Sweden in 1931–34, and studies on the degree of attack in different varieties of wheat. Medd. Vaxtskyddsanst (Stockholm) No. 10. 1935.
380. MUMFORD, E. P. Cotton stainers and certain other sap feeding insect pests of the cotton plant. 79 pp. 1926.
381. ————. On the curly top disease of the sugar beet: A biochemical and histological study. Ann. Appl. Biol. **17**: 28–35. 1930.
382. ————. Studies in certain factors affecting the resistance of plants to insect pests. Science **73**: 49–50. 1931.
383. ————, AND HEY, D. H. The water balance of plants as a factor in their resistance to insect pests. Nature **125**: 411–412. 1930.
384. NAKAYAMA, S. Observations on the injury of *Grapholitha glycinivorella* Mats., and varieties of soy beans. [In Japanese.] Jour. Plant. Prot. **26**: 868–875, Tokyo, 1939. [Abstracted from Rev. Appl. Ent.]
385. NEISWANDER, C. R., AND HUBER, L. L. Height and silking as factors influencing European corn borer population. Ent. Soc. Amer. Ann. **22**: 527–542. 1929.
386. NEWCOMER, E. J., YATHERS, M. A., AND WHITCOMB, W. D. Control of the codling moth in the Pacific Northwest. U. S. Dept. Agr. Farmers' Bul. 1326. 1924.
387. NOBLE, W. B., CARTWRIGHT, W. B., AND SUNESON, C. A. Inheritance of resistance to Hessian fly. Jour. Econ. Ent. **33**: 580–581. 1940.
388. NORTH, H. F. A., AND THOMPSON, G. A., JR. Investigations regarding blue grass webworms in turf. Jour. Econ. Ent. **26**: 1117–1125. 1933.
389. NOUGARET, R. L., AND LAPHAM, MACY H. A study of Phylloxera infestation in California as related to types of soils. U. S. Dept. Agr. Tech. Bul. 20. 1928.
390. OKADA, I. Oviposition of *Grapholitha glycinivorella* Mats. in relation to some legumes. [In Japanese.] Oyo Dobuts. Zasshi **19** (1): 8–17. 1938.
391. OSHIMA, MASAMITSU. Formosan termites and methods of preventing their damage. Philippine Jour. Sci. **15** (4): 319–383. 1919.
392. PACKARD, A. S. The Hessian fly. Its ravages, habits and means of preventing its increase. U. S. Ent. Comm. Rpt. 3. 198–248. 1883.
393. PACKARD, C. M. The Hessian fly in California. U. S. Dept. Agr. Tech. Bul. 81. 1928.
394. ————. Breeding wheat and alfalfa for resistance to insect attack. [Manuscript for publication in the Jour. Econ. Ent. **34**: 1941.]
395. PAINTER, REGINALD H. Notes on the injury of plant cells by chinch bug feeding. Ent. Soc. Amer. Ann. **21**: 232–242. 1928.
396. ————. Observations on the biology of the Hessian fly. Jour. Econ. Ent. **23**: 326–328. 1930.
397. ————. The food of insects and its relation to resistance of plants to insect attack. Amer. Nat. **70**: 547–566. 1936.
398. ————. The economic value and biologic significance of plant resistance to insect attack. [Manuscript for publication in the Jour. Econ. Ent. **34**: 1941.]
399. ————, AND BRUNSON, ARTHUR M. Differential injury within varieties, inbred lines, and hybrids of field corn caused by the corn earworm, *Heliothis armigera* (Hbn.). Jour. Agr. Res. **60**: 81–100. 1940.
400. ————, ————, AND PARKER, J. H. The resistance of crop plants to insect injury. Kans. Agr. Exp. Sta. Bien. Rpt. 6. 1930–1932: 99. 1932.
401. ————, AND GRANDFIELD, C. O. A preliminary report on resistance

of alfalfa varieties to pea aphids, *Illinoia pisi* (Kalt.). Jour. Amer. Soc. Agron. **27**: 671–674. 1935.
402. ———, Jones, Elmer T., Johnston, C. O., and Parker, John H. Transference of Hessian fly resistance and other characteristics of Marquillo spring wheat to winter wheat. Kans. Agr. Exp. Sta. Tech. Bul. 49. 1940.
403. ———, Salmon, S. C., and Parker, John H. Resistance of varieties of winter wheat to Hessian fly, *Phytophaga destructor* Say. Kans. Agr. Exp. Sta. Tech. Bul. 27. 1931.
404. ———, Snelling, Ralph O., and Brunson, Arthur M. Hybrid vigor and other factors in relation to chinch bug resistance in corn. Jour. Econ. Ent. **28**: 1025–1030. 1935.
405. Pal, B. P. A note on the relation between the internal stem structure of certain varieties of gram (*Cicer arietinum* L.) and their resistance to cutworm attack. Proc. Indian Acad. Sci. (Pusa) **3** (6): 527–534. 1936.
406. Parker, John H. Insect resistance in wheats and sorghums—a heritable character. U. S. Dept. Agr. Yearbook in 1931. 316–317. 1931.
407. ———. Wheat improvement in Kansas, 1874–1934. Kans. State Board Agr. Bien. Rpt. 29. 156–157. 1935.
408. ———, and Painter, R. H. Insect resistance in crop plants. Sixth Int. Cong. Gen. Proc. **2**: 150–152. 1932.
409. Parkin, E. A. Vessel diameter in relation to *Lyctus* attack. Empire Forest Jour. **12**: 266. 1933.
410. ———, and Phillips, E. W. J. The depletion of starch from timber in relation to attack by *Lyctus* beetles. IV. A third experiment on the girdling of standing oak trees. Forestry **13**: 134–145. 1939.
411. Parnell, F. R. The breeding of jassid-resistant cotton in South Africa. Empire Cotton Growing Corp., Rpts. Exp. Stas. 1923–1925: 5–9. 1925.
412. ———. Cotton breeding in the low veld. Jour. Dept. Agr. South Africa **11**: 153–158. 1925.
413. ———. The breeding of jassid-resistant cottons. Report for season 1924–25. Empire Cotton Growing Rev. **2**: 330–336. 1925.
414. ———. South Africa: Cotton-breeding station, Barberton. Report for season 1925–26. Empire Cotton Growing Corp. Rpts. Exp. Stas., 1925–26. 24–25. 1927.
415. ———. Report on the work of the cotton-breeding station, Barberton, Transvaal, for the season 1926–27. Empire Cotton Growing Corp. Rpts. Exp. Stas. 1926–27. 49–65. 1928.
416. ———. Plant breeding and cotton insect pests. In third conference on cotton growing problems. Report and summary of proceedings. Empire Cotton Growing Corp. (London) 81–85. 1938.
417. Parr, T. J. *Matsucoccus* sp. A scale insect injurious to certain pines in the Northeast (Hemiptera-Homoptera). Jour. Econ. Ent. **32**: 624–630. 1939.
418. Pasquier, Du. Le coton aux Indes Anglaises. (British India cotton.) Bul. Econ. Indochine. **30** (187): 297–305. 1927. [Abstracted from Biol. Abst.]
419. Patch, L. H. Resistance of a single cross hybrid strain of field corn to European corn borer. Jour. Econ. Ent. **30**: 271–278. 1937.
420. ———. Some factors determining corn borer damage. Jour. Econ. Ent. **22**: 174–183. 1939.
421. ———, Bottger, G. T., and App, B. A. Comparative resistance to the European corn borer of two hybrid strains of field corn at Toledo, Ohio. Jour. Econ. Ent. **31**: 337–340. 1938.
422. Pemberton, C. E. 31–1389, its susceptibility to insect attack in Hawaii. Hawaii Plant Rec. **43**: 254. 1939.

423. PERSON, HUBERT L. Theory in explanation of the selection of certain trees by the western pine beetle. Jour. For. **29**: 696–699. 1931.
424. PHILLIPS, W. J., AND BARBER, GEORGE W. The corn earworm as an enemy of field corn in the Eastern States. U. S. Dept. Agr. Farmers' Bul. 1651. 1931.
425. ———, AND ———. The value of husk protection to corn ears in limiting corn earworm injury. Va. Agr. Exp. Sta. Tech. Bul. 43. 1931.
426. ———, AND KING, KENNETH M. The corn earworm, its ravages on field corn and suggestions for control. U. S. Dept. Agr. Farmers' Bul. 1310. 1923.
427. PHIPPS, C. R., AND DIRKS, C. O. Notes on the biology of the apple maggot. Jour. Econ. Ent. **26**: 349–358. 1933.
428. PIETERS, A. J. Red clover's hairiness in American types due to the leaf hopper. U. S. Dept. Agr. Yearbook 1928. 521–524. 1929.
429. POOLE, CHARLES F. Corn earworm resistance in maize varieties at Davis, California, 1934. Amer. Soc. Hort. Sci. Proc. 1934 **32**: 453–457. 1935.
430. ———. New sweet corns resistant to earworm. Amer. Soc. Hort. Sci. Proc. 1935. **33**: 496–501. 1936.
431. ———. Resistance to corn earworm injury in the Charleston, South Carolina, area. Amer. Soc. Hort. Sci. Proc. 1936. **34**: 566–569. 1937.
432. POOS, F. W., AND JOHNSON, H. W. Injury to alfalfa and red clover by the potato leafhopper. Jour. Econ. Ent. **29**: 325–331. 1936.
433. ———, AND SMITH, FLOYD F. A comparison of oviposition and nymphal development of *Empoasca fabae* (Harris) on different host plants. Jour. Econ. Ent. **24**: 361–371. 1931.
434. POPOV, V. I. Varieties of maize with long, thick and close-fitting husks are much less severely damaged with *Heliothis obsoleta* F. Observations on the corn earworm *Heliothis obsoleta* Fab. (*H. armigera* Hubn.) in Obrastzovtchiflik. [In Bulgarian.] Zeits. Landw. Vers. Sta. Bulg. **6** (3–4): 53–75. 1934.
435. PRIESNER, H. A brief note on the relation between the physiological condition of the plant and insect attack. Fouad 1er Ent. Soc. Bul. 1938. **22**: 279–283. 1939.
436. PRUTHI, H. S., AND NARAYANAN, E. S. A study of the behavior of some common varieties of sugarcane in reference to the attack of borers at Pusa (Bihar) during 1935–39. Indian Nat. Inst. Sci. Proc. **4**: 87–107. 1938.
437. RAHMAN, K. A., AND BHARDWAJ, N. K. The grape-vine thrips (*Phipiphorothrips cruentetus* Hd.) (*Thripidae, Terebrantia*). Indian Jour. Agr. Sci. **7**: 633–651. 1937.
438. RAIBOI, D. D. The self-protection of fruits. [Abstract from original Russian in Rev. Appl. Ent. **3**: 484. 1915.]
439. RASMUSON, ——. Inheritance in Vitis. Mitt K. Biol. Anst. Landu Forstw. **15**: 28–34. 1914. [Abstracted from Exp. Sta. Record.]
440. RAUCOURT, M., AND TROUVELOT, B. Les principes constituants de la pomme de terre et le doryphore. Réactions d'ordre sensitif chez la larve. Ann. Epith. Phytogen. (N. S.) **2** (1): 51–98. 1936.
441. RILEY, C. V., PACKARD, A. S., JR., AND THOMAS, CYRUS. The rocky mountain locust. U. S. Ent. Comm. Ann. Rpt. 1. 252. 1877.
442. ROACH, W. A. Studies on possible causes of immunity. Ann. Appl. Biol. **24**: 206–210. 1937.
443. ———, AND MASSEE, A. M. Preliminary experiments on the physiology of the resistance of certain rootstocks to attack by woolly aphis. East Malling (Kent) Res. Sta. Ann. Rpt. Sup. II. 1928–1930. 111–120. 1931.
444. ROBERTS, I. P., SLINGERLAND, M. V., AND STONE, J. L. The Hessian

fly, its ravages in New York in 1901. Cornell Agr. Exp. Sta. Bul. 194: 239–260. 1901.

445. ROCKWOOD, L. P., AND REEHER, MAX M. The Hessian fly in the Pacific Northwest. U. S. Dept. Agr. Tech. Bul. 361. 1933.

446. RONEY, J. N. Truck crop insect investigations. Tex. Agr. Exp. Sta. Ann. Rpt. 46. 50. 1933.

447. ROUBAUD, E. Sur les(préférences trophiques de la Pyrale du Mais (*Pyrausta nubilalis*). Importance de l'armoise commune (*Artemisia vulgaris*) comme plante d'élection déviatrice, pour la protection des cultures. Comp. Rend. Acad. Sci. Fr. **185**: 1158–1160. 1927. [Abstracted from Rev. Appl. Ent.]

448. ————. Biological researches on *Pyrausta nubilalis* Hb. Int. Corn Borer Invest. Sci. Rpts. 1927–28. 1–40. Int. Live Stock Exp. Chicago, Ill. 1928.

449. ————. Biological researches on *Pyrausta nubilalis* Hb. Int. Corn Borer Invest. Sci. Rpt. 2. 1–21. Int. Live Stock Exp. Chicago, Ill. 1929.

450. ————. Biological researches on *Pyrausta nubilalis.* Int. Corn Borer Invest. Sci. Rpt. 3. 1–27. Int. Live Stock Exp. Chicago, Ill. 1930.

451. SAKHAROV, N. L. The sunflower moth in connection with the cultivation of a resistant sunflower, *Homoeosoma nebulella* Hb. Novaya Derevnya. Moscow, 1925. [With English summary.] [Abstracted from Rev. Appl. Ent.]

452. ————. The resistance of agricultural plants, immunity and agricultural measures in the control of pests. [In Russian.] Social. Grain Fmg. (Saratov) **5** (1): 147–156. 1935.

453. ————. Wheat pests. [In Russian.] Social. Grain Fmg. **5** (6): 151–165. 1936.

454. SALMEN, J. Eine gegen die Blutlaus unempfindliche Apfelsorte. (A variety of apple immune to the woolly aphis.) Wiener landw. Ztg. **61**: 269. 1921. [Abstract in Zeits. Pflanzenkr. Gallenkunde **32** (1–2): 81. 1922).] [Abstracted from Rev. Appl. Ent.]

455. SALMON, S. C., SWANSON, C. O., AND LAUDE, H. H. Blackhull wheat in Kansas. Kans. Agr. Exp. Sta. Bul. 241. 1927.

456. SALTER, R. M., THATCHER, L. E., AND MCCLURE, J. T. Agronomic research on the European corn borer in Ohio. Jour. Amer. Soc. Agron. **20**: 1011–1032. 1928.

457. SAPEHIN, A. A. Hylogenetic investigations of the Vulgare group in Triticum. Bul. Appl. Bot., Genet. & Pl. Breed. **29**: 127–166. [English summary 161–166.] 1928.

458. ————. Hylogenetics of durum wheat. Bul. Appl. Bot., Genet. & Pl. Breed. **29**: 167–224. [English summary 221–224.] 1928.

459. SCHAFER, P. Researches and problems connected with the control of the Colorado beetle by breeding. Zeits. Pflanzenzüchtung **23**: 239–322. 1939.

460. SCHLOSBERG, MORRIS, AND BAKER, W. A. Sweet corn resistant to larval survival of the European corn borer. Jour. Econ. Ent. **32**: 530–534. 1939.

461. ————, AND MATHES, RALPH. Egg and larval populations of European corn borer. Jour. Econ. Ent. **30**: 280–287. 1937.

462. SCHOENE, W. J., AND UNDERHILL, G. W. Resistance of certain varieties of apple trees to injury by the leafhopper, *Empoasca fabae.* Va. Agr. Exp. Sta. Tech. Bul. 59. 1937.

463. SCHWARTZE, C. D., AND HUBER, GLEN A. Aphid resistance in breeding mosaic escaping red raspberries. Science **86** (2224): 158–159. 1937.

464. ————, AND ————. Further data on breeding mosaic-escaping raspberries. Phytopath. **29**: 647–648. 1939.

465. SCHWARTZ, M., AND MÜLLER-BÖHME, H. Investigations on the capacity for resistance to *Leptinotarsa decemlineata* Say, of wild species of plants allied to the potato and of crosses between them and cultivated potato. (Addresses at the Plant Protection Meeting of the German Imperial Biological Institute, February 1938.) Mitt. Biol. Reichsanst., pt. 58: 47–54. 1938.
466. SEAMANS, H. L., AND MCMILLAN, ELLIS. The effect of food plants on the development of the pale western cutworm, *Agrotis orthogonia* Morr. Jour. Econ. Ent. **28**: 421–425. 1935.
467. SEARLS, ED. M. A preliminary report of the resistance of certain legumes to certain Homopterous insects. Jour. Econ. Ent. **25**: 46–49. 1932.
468. ———. The relation of foliage color to aphid resistance in some varieties of canning peas. Jour. Agr. Res. **51**: 613–619. 1935.
469. ———. Time of fumigation of aphid infested canning peas and resistance of the plants to subsequent infestations. Jour. Econ. Ent. **32**: 90–93. 1939.
470. SHARPLES, A. The laticiferous system of *Hevea brasiliensis* and its protective function. Ann. Bot. **32** (126): 247–251. 1918.
471. SHEN, T. H., AND SHEN, H. N. Breeding rice in China for resistance to the stem borer. Coll. Agr. & For., Univ. Nanking, Bul. 20 (N. S.). 1934.
472. SHULL, WESLEY EARL. An investigation of the Lygus species which are pests of beans (Hemiptera, Miridae). Ida. Agr. Exp. Sta. Res. Bul. 11. 1933.
473. SISON, PEDRO. The slug caterpillar on Abaca, *Thosea sinensis* Wlk. Its life history and habits as observed on Davao, and suggestions for control. Philippine Jour. Agr. **3**: 163–186. 1932.
474. SKAIFE, S. H. Pea and bean weevils. Union So. Afr. Dept. Agr. Bul. 12. 1918.
475. SLEESMAN, J. P. Onion maggot, *Hylemyia antiqua* Meig. Ohio Agr. Exp. Sta. Bul. 532 (52nd Ann. Rpt.). p. 41. 1934.
476. ———. Tests on the susceptibility of varieties of onions to *Thrips tabaci* Lind. Ohio Agr. Exp. Sta. Bul. 548 (53rd Ann. Rpt.). 39–45. 1935.
477. ———. Influence of variety on potato leafhopper population. Ohio Agr. Exp. Sta. Bul. 561 (54th Ann. Rpt.). p. 43. 1936.
478. ———. Resistance in wild potatoes to attack by the potato leafhopper and the potato flea beetle. Amer. Pot. Jour. **17**: 9–12. 1940.
479. ———, AND BUSHNELL, JOHN. Variations in nymphal populations of the potato leafhopper on different varieties of potatoes. Amer. Pot. Jour. **14**: 242–245. 1937.
480. SMITH, FLOYD F., AND POOS, F. W. The feeding habits of some leaf hoppers of the genus *Empoasca*. Jour. Agr. Res. **43**: 267–285. 1931.
481. SMITH, GEORGE D. Studies in the biology of the Mexican cotton boll weevil on short staple upland, long staple upland, and sea-island cottons. U. S. Dept. Agr. Bul. 926. 1921.
482. SNELLING, RALPH O. Third generation and method of migration of chinch bug in southwestern Oklahoma. Jour. Econ. Ent. **29**: 797–803. 1936.
483. ———. The place and methods of breeding for insect resistance in cultivated plants. [Manuscript for publication in the Jour. Econ. Ent. **34**: 1941.]
484. ———. Breeding corn for insect resistance. Nat. Seedsman **6** (6): 24. 1940.
485. ———, BLANCHARD, RALPH A., AND BIGGER, JOHN H. Resistance of corn strains to the leaf aphid, *Aphis maidis* Fitch. Jour. Amer. Soc. Agron. **32**: 371–381. 1940.
486. ———, AND DAHMS, REYNOLD G. Resistant varieties of sorghum

and corn in relation to chinch bug control in Oklahoma. Okla. Agr. Exp. Sta. Bul. 232. 1937.

487. ———, PAINTER, REGINALD H., PARKER, JOHN H., AND OSBORN, W. M. Resistance of sorghums to the chinch bug. U. S. Dept. Agr. Tech. Bul. 585. 1937.

488. SNYDER, T. E. Biology of the termites of the eastern United States with preventive and remedial measures. U. S. Dept. Agr. Bur. Ent. Bul. 94. Part 2. 1915.

489. ———. White-ant proof wood for the tropics. Jour. Econ. Ent. **14**: 496–501. 1921.

490. SPEYER, W. Blutlausbekämpfung durch Auswahl geeigneter Apfelsorten. (Woolly aphis control by selection of suitable apple varieties.) Provinz Monatsschr. Obst. Wein u. Gartenbau. **24**: 40–41. 1923. [Abstracted from Rev. Appl. Ent.]

491. SPINKS, G. T. Families of strawberry seedlings bred for resistance to aphis. Agr. & Hort. Res. Sta. (Univ. Bristol) Ann. Rpt. 1929. 17–27. 1929.

492. SPITTAL, J. P. Varietal susceptibility of the apple to various injuries. Acadian Ent. Soc. Proc. No. 10: 17–22. 1924.

493. SQUIRE, F. A. A note on the resistance of *Hibiscus esculentus* and *Gossypium trilobum* to *Platyedra gossypiella* Saund. Emp. Cotton Growers Rev. **16**: 268–271. 1939.

494. STANILAND, L. N. The immunity of apple stocks from woolly aphis. Jour. Pomol. & Hort. Sci. **3**: 85–95. 1923.

495. ———. The immunity of apple stocks from attacks of woolly aphis, *Eriosoma lanigerum* Hausmann. Part II. The causes of the relative resistance of the stocks. Bul. Ent. Res. **15**: 157–170. 1924.

496. STEINER, L. F. Observations on the distribution of hibernating oriental fruit worms. Jour. Econ. Ent. **25**: 864–868. 1932.

497. STELLWAAG, F. Die Grundlagen für den Anbau reblauswiderstandsfähiger Unterlagsreben zur Immunisierung verseuchter Gebiete. (The basis for the cultivation of stocks resistant to Phylloxera for the immunization of infested districts.) Monogr. Angew. Ent. 7. 1924. [Abstracted from Rev. Appl. Ent.]

498. STOREY, G. The present situation with regard to the pink boll worm in Egypt. Min. Agr. Egypt Bul. 16. 1921.

499. STRICKLAND, E. H. Relative susceptibility of wheat varieties to wireworm damage. Sci. Agr. **12**: 88–91. 1931.

500. SUMMERVILLE, W. A. T. Notes on the onion thrips. Queensland Agr. Jour. **39**: 41–46. 1933.

501. SWEETMAN, HARVEY L. The biological control of insects. 461 pp. 1936.

502. TAKANO, S. Relation of sugar cane varieties to insect pests. [In Japanese.] Formosan Sug. Plant. Assoc. Jour. **15**: 195–198. 1937.

503. TCHESNOKOV, P. G. [See Chesnokov, P. G. This name has been shown in Rev. Appl. Ent. as Chesnokov and in Biol. Abst. as Tchesnokov.]

504. THEOBALD, F. V. The woolly aphid of the apple and elm. Part I. Jour. Pomol. **2** (1920–1921).

505. THOMAS, C. A. The biology and control of wireworms. A review of the literature. Pa. Agr. Exp. Sta. Bul. 392. 1940.

506. THOMAS, EDWARD L. The influence of temperature, moisture, and food upon the development and survival of the saw-toothed grain beetle. Jour. Agr. Res. **60**: 605–615. 1940.

507. THOMAS, F. L., MILLS, H. B., AND JONES, S. E. Varietal resistance (Cotton flea hopper). Tex. Agr. Exp. Sta. Ann. Rpt. 46. 1933.

508. ———, GAINES, J. C., AND JANES, M. J. The cotton flea hopper, varietal resistance. Texas Agr. Exp. Sta. 51st Ann. Rpt. 1938. 1938.

509. THOMAS, HAROLD E. The production of strawberries in California. Calif. Agr. Exp. Sta. Cir. 113. 1939.
510. THOMPSON, B. G. The peach and prune root borer injurious to cherries. Jour. Econ. Ent. **19**: 779–780. 1926.
511. THOMPSON, R. W. Notes on corn borer resistance in hybrid corn. Ent. Soc. Ontario Ann. Rpt. 1937. **68**: 28–32. 1938.
512. ———. Further notes on corn borer resistance in hybrid corn; with a brief statement of the investigation situation in Ontario in 1938. Ent. Soc. Ont. Ann. Rpt. 1938. **69**: 76–81. 1939.
513. THORPE, W. H. Biological races in insects and allied groups. Biol. Rev. & Biol. Proc. Cambridge Phil. Soc. **5** (3): 177–212. 1930.
514. TOPI, M. Sulle probabili cuase del diverso comportamento della fillossera, specialmente gallecola, in rapporto ai vari vitigni americani. (Cause of different behavior of leaf-gall Phylloxera on various American grape-vines.) Monitore Zool. Ital. **37**: 74–84. 1926. [Abstracted from Rev. Appl. Ent.]
515. ———. Sulla esistenza di diverse razze della fillossera della vite e sui loro presunti caratteri distintivi. (On the existence of various races of vine Phylloxera and on their presumed differential characters.) Monitore Zool. Ital. **38**: 167–180. 1927. [Abstracted from Rev. Appl. Ent.]
516. ———. Ulteriori ricerche sulla esistenza di razze diverse della fillossera della vite. (Races in grape Phylloxera.) Bul. R. Staz. Patol. Veg. **9**: 75–92. 1929. [Abstracted from Biol. Abst.]
517. TRAGARDH, I. On the chematropism of insects and its significance for economic entomology. Bul. Ent. Res. **4**: 113–117. 1913.
518. TREHERNE, R. C. The natural immunity or resistance of plants to insect attack. Agr. Gaz. Canada **4**: 855–859. 1917.
519. TROUVELOT, B., AND BREJOUX, H. Rapports biologiques entre le doryphore *Leptinotarsa decemlineata* Say et *Solanum demissum*. Somnolence et reduction d'activité presentées par les larves consommant les feuilles de cette plante. Rev. Path. Veg. Ent. Agr. **26**: 142–147. 1939.
520. ———, GRISON, AND DIXMERAS. Remarques sur les différences d'attaque par le doryphore de variétés courantes de pommes de terre. Comp. Rend. Acad. Agr. Fr., Paris **22** (13): 513–517. 1936.
521. ———, LACOTTE, DUSSY, AND THENARD. Les qualités élémentaires des plantes nourricières du *Leptinotarsa decemlineata* Say et leur influence sur le comportement de l'insecte. Comp. Rend. Acad. Sci. Paris **197**: 355–356. 1933. [Abstracted from Rev. Appl. Ent.]
522. ———, MÜLLER-BÖHME, H., AND LACOTTE. Remarques sur le comportement du doryphore, *Leptinotarsa decemlineata* Say, sur des hybrides *Solanum demissum-Solanum tuberosum*. Rev. Path. Veg. Ent. Agr. **25**: 273–276. 1938.
523. ———, AND THENARD, J. Remarques sur les éléments des végétaux contribuant à limiter ou à empêcher la pullulation du *Leptinotarsa decemlineata* Say sur de nombreuses espèces on races végétales. Rev. Path. Veg. Ent. Agr. **18**: 277–285. 1931.
524. TSA, P. H. A report on the rice variety test for paddy borer. Agr. Coll. Univ. Chekiang, Bul. 9. 1930.
525. TUCKER, R. W. E. Varietal factors in cane which may influence extent of oviposition by *Diatraea saccharalis* and a possible method for determining varietal susceptibility to borer attack. Jour. Dept. Sci. Agr., Barbados **2**: 53–59. 1933.
526. ———. A preliminary investigation into cane varieties and infestation by *Diatraea saccharalis*. Agr. Jour. Barbados **5**: 121–142. 1936.
527. ———. Some aspects of the control of the sugar cane moth borer, *Diatraea saccharalis* F. Int. Soc. Sug. Cane Tech. Proc. **6**: 240–243. 1939.

528. Twight, E. H. Resistant vines and their hybrids. Calif. Agr. Exp. Sta. Bul. 148. 1902.
529. Twinn, C. R. Observations on the host selection habits of *Pieris rapae* L. Ontario Ent. Soc. Ann. Rpt. 55. 1924. 75–80. 1925.
530. Tydeman, H. M. Apple rootstocks immune from woolly aphis. East Malling (Kent) Res. Sta. Ann. Rpt. 1934. 115–122. 1935.
531. Tytler, Elmer. Hessian fly. Encyclopedia Brittanica 8. 1797.
532. Underhill, G. W., and Cox, James A. Studies on the resistance of apple to the woolly apple aphid, *Eriosoma lanigerum* (Hausm.). Jour. Econ. Ent. **31**: 622–625. 1938.
533. Van Dine, D. L. The sugar cane insects of Hawaii. U. S. Dept. Agr. Bur. Ent. Bul. 93. 1911.
534. Verschaffelt, E. The causes determining the selection of food in some herbivorous insects. Kon. Akad. Wetensk (Amsterdam) Proc. **13**: 536. 1910.
535. Vereshchagin, V. A. Susceptibility of spring wheat of different sowing terms of *Oscinosoma frit* L. attacks in the district of western Siberia. [In Russian.] Plant Prot. **2**: 45–56. 1935.
536. Vouk, V. A corn variety test under artificial corn borer infestation. Int. Corn Borer Inv. Sci. Rpt. 3. 116–120. Int. Livestock Exp., Chicago, Ill. 1930.
537. Wadley, F. M. Ecology of *Toxoptera graminum*, especially as to factors affecting importance in northern United States. Ent. Soc. Amer. Ann. **24**: 325–395. 1931.
538. Wakeland, C. Resistance by certain onion varieties to onion thrips. Idaho Agr. Exp. Sta. Bul. 220. 1936.
539. Walker, H. G., and Anderson, L. D. Sweet corn variety tests in relation to corn earworm damage at Norfolk, Virginia. Va. Truck Crop Exp. Sta. Bul. 100: 1581–1593. 1938.
540. Walsh, Benj. D. On phytophagic varieties and phytophagic species. Ent. Soc. Philad. Proc. **5**: 194–216. 1865.
541. Walter, E. V., and Brunson, Arthur M. Differential susceptibility of corn hybrids to *Aphis maidis*. Jour. Econ. Ent. **33**: 623–628. 1940.
542. Walther, E. Genetic constitution of host plant as a factor in pest control. Calif. Dept. Agr. Mo. Bul. 24: 242–244. 1935.
543. Wardle, R. A. The problems of applied entomology. 587 pp. 1929.
544. ————, and Buckle, P. The principles of insect control. 295 pp. 1923.
545. Ware, J. O. Plant breeding and the cotton industry. U. S. Dept. Agr. Yearbook. 657–744. 1937.
546. Watts, J. G. Thrips (*Sericothrips variabilis* Beach) on seedling cotton. S. C. Agr. Exp. Sta. 49th Ann. Rpt. 1935–36. 45–49. 1936.
547. ————. Thrips on seedling cotton. S. C. Agr. Exp. Sta. 50th Ann. Rpt. 1936–37. 54–57. 1937.
548. Webster, F. M. Does the wheat stem maggot, *Meromyza americana*, discriminate between different varieties of wheat? Insect Life **2**: 87. 1889.
549. Webster, R. L. The apple leafhopper. Iowa Agr. Exp. Sta. Bul. 111. 1910.
550. ————. Insect tolerance. Jour. Econ. Ent. **26**: 1016–1021. 1933.
551. ————. Arsenic deposit and codling moth control. Jour. Econ. Ent. **27**: 410–417. 1934.
552. Whitcomb, W. D. The apple leaf-curling midge, a new pest of apples. Jour. Econ. Ent. **27**: 355–361. 1934.
553. Wilcox, R. B., and Beckwith, C. S. A factor in the varietal resistance of cranberries to false-blossom disease. Jour. Agr. Res. **47**: 583–590. 1933.
554. Willcocks, F. C. Insects and related pests of Egypt. (Pink boll worm.) Sultanic Agr. Soc. 1 (1): 284–285. 1916.

555. Williams, Francis X. Cane borer, *Rhabdocnemis obscura.* Rpt. Exp. Sta. Hawaii Sug. Pl. Assn. 20–21. 1935.
556. ———. Hard canes (sugar) are more resistant to attack by the borer, *Rhabdocnemis obscura* Boisd. Rpt. Exp. Sta. Hawaii Sug. Pl. Assn. 17–25. 1935.
557. Wilson, S. E. Changes in the cell contents of wood (xylem parenchyma) and their relationships to the respiration of wood and its resistance to *Lyctus* attack and to fungal invasion. Ann. Appl. Biol. **20**: 661–690. 1933.
558. Winter, J. D. A preliminary account of the raspberry aphids. Minn. Agr. Exp. Sta. Tech. Bul. 61. 1929.
559. Withycombe, C. L. Studies on the aetiology of sugar cane froghopper blight in Trinidad. Ann. Appl. Biol. **13**: 64–108. 1926.
560. Wolcott, George N. The influence of the variety of sugar-cane on its infestation by *Diatraea saccharalis,* and other factors affecting the abundance of the moth borer. Jour. Dept. Agr. Porto Rico **6**: 21–31. 1922.
561. ———. Haitian cotton and the pink boll worm. Bul. Ent. Res. **18**: 79–82. 1927.
562. ———. The pink boll worm in Haiti. Fourth Int. Cong. Ent. Trans. **2**: 68–72. Ithaca. 1928.
563. Woodworth, C. W. Variations in Hessian fly injury. Calif. Agr. Exp. Sta. Rpt. 1890. 312–318. 1891.
564. Worrall, Lloyd. Jassid-resistant cotton. Jour. Union So. Afr. Dept. Agr. **7**: 225–228. 1923.
565. ———. A jassid-resistant cotton. Jour. Dept. Agr. So. Afr. **10**: 487–491. 1925.
566. Yamasaki, M., and Arikado, H. The cell-sap concentration of sugar-cane varieties in relation to their resistance to the attack of the white leaf louse, *Oregma lanigera,* Zehnt. Int. Soc. Sug. Cane Tech. Proc. **6**: 475–478. 1939.
567. ———, ———. Resistance of sugar cane varieties to *Ceratovacuna lanigera* Zehnt. and the concentration of the cell sap. [In Japanese.] Jap. Ass'n Adv. Sci. Rpt. **14** (1): 153–155. 1939.

THE BOTANICAL REVIEW

VOL. VII NOVEMBER, 1941 No. 11

PLASTID PIGMENTS

WITH SPECIAL REFERENCE TO THEIR PHYSICAL AND PHOTOCHEMICAL PROPERTIES AND TO ANALYTICAL METHODS

F. P. ZSCHEILE

Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Indiana

CONTENTS

INTRODUCTION

During the past six years, several excellent reviews which have appeared deal with certain phases of the field of plant pigments. These have been quite detailed, especially from the chemical viewpoint. In this review, only a skeleton outline of chemical properties, sufficient for a discussion of physical properties, will be presented. Most emphasis will be placed upon physical and photochemical properties and upon their significance for a better understanding of the function and behavior of the green and yellow fat-soluble plastid pigments. Photometric analytical methods are stressed because of the recent rapid growth of interest in their application to physiological problems.

No attempt is made to include references to all work relating to plastid pigments reported in the last few years, but instead it is proposed to review briefly the more important papers which recently have come to the writer's attention, and which relate to the limited field discussed above. The divisions of subject matter necessarily overlap somewhat because of interrelations between them.

CAROTENOIDS

Chemical Relationships and Reactions

The carotenoids comprise a large group of compounds, the most important of which are essentially modifications of the two molecular structures indicated on page 590.[1]

These were chosen because they occur abundantly in plants and may serve as illustrations for our very short consideration of organic constitution. Carotenoids are nitrogen-free polyene pigments consisting of a long acyclic chain (III) of carbon atoms joined in an uninterrupted sequence of conjugated double bonds. This chain is terminated at both ends by groups which may or may not be cyclized (I and II) and which have isomeric forms. Groups I and and II may be different in the same molecule. Carotenoids are divided into several groups:[2] the hydrocarbons, such as lycopene

[1] This system of numbering the carbon atoms is employed by Karrer and by Bogert (19).

[2] The nomenclature of carotenoids employed in this paper follows the suggestions of Peterson, Hughes, and Payne (213). They suggest the use of the term "carotenol" to indicate a carotenoid which contains oxygen in a hydroxyl group. The older designation of "xanthophyll" expresses nothing concerning the chemical nature of this group of compounds. The terms "carotene" and "carotenoid" are named after the cultivated carrot, *Daucus*

and the carotenes [A], and the related oxygenated compounds, represented by the carotenols [B] (formerly known as xanthophylls or phytoxanthins, and which are hydroxyl derivatives of carotenes) and the esters, ethers, ketones, hydroxyketones and acids.

The first formula [A] illustrates the *beta* structure, in which each terminal ring has a double bond in conjugation with the long conjugated aliphatic chain. The second formula [B] illustrates the *alpha* structure, characterized by the fact that in one ring the double bond lies between carbon atoms 4′ and 5′ rather than between 5′ and 6′. It is thus removed from the conjugated chain, and this causes rather large differences in physical properties.

The chemical structure of other more commonly occurring carotenoids will be described briefly with reference to the above illustrations. Rings are closed unless otherwise noted.

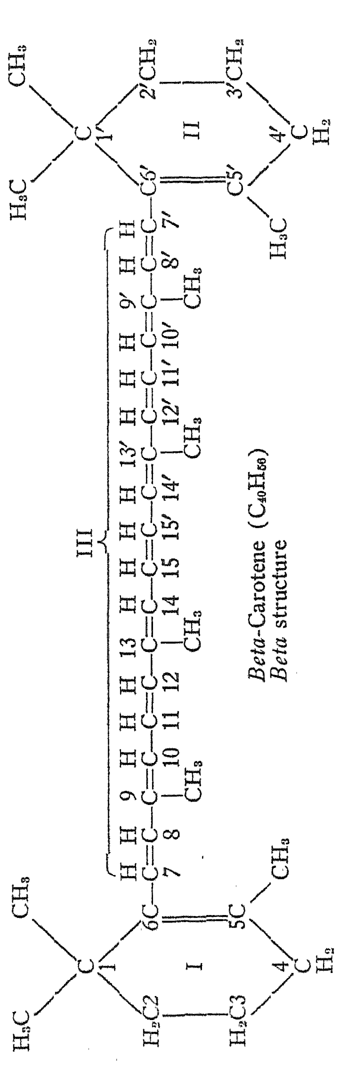
1. *Alpha*-carotene [B]. No – OH groups.
2. Cryptoxanthol [A]. One – OH group on carbon 3′.
3. Zeaxanthol [A]. – OH group on 3 and 3′.
4. Lycopene [A]. Rings broken between carbons 1 and 6, and between 1′ and 6′. Double bonds between 1 and 2, and between 1′ and 2′.

---

carota, the root of which was an early source of yellow pigments and which contains, in appreciable quantity, only three of the sixty or more carotenoid pigments known today. These names have little chemical significance, but universal usage has made them acceptable. Some specific names of carotenoids originated from the name of the plant source from which the pigment was first isolated or in which it is unusually abundant. For instance, "zeaxanthin" indicates a pigment found in seeds of plants belonging to the *Zea* genus. Actually, as these writers point out, the suffix "–in" is by international agreement reserved for substances of unknown chemical nature, and therefore it is no longer suitable for compounds whose molecular structure has been satisfactorily determined. The suffix "–ol" on a carotenoid name indicates the presence of a hydroxyl group on a carotenoid structure.

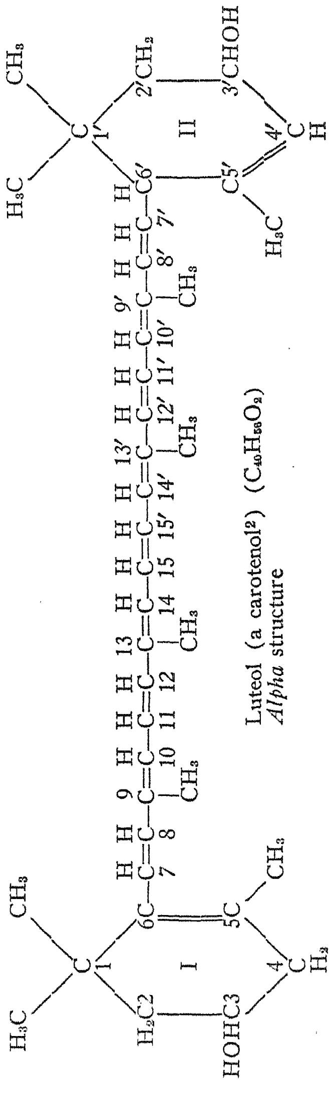
It seems reasonable to retain the customary manner of distinguishing between isomers by *alpha, beta,* and *gamma* designations. Brevity and common usage are the only points in favor of this, however. The chemical name, in which the particular carbon having the group which is additional to the hydrocarbon carotenoid nucleus is specifically designated, tells more about molecular structure. Cryptoxanthol would thus be named 3-hydroxy-*beta*-carotene and luteol would be 3–3′-dihydroxy-*alpha*-carotene. However, such names are too long for convenient reference. Because of these considerations, we shall follow the suggestion originally credited by Peterson, Hughes and Payne (213) to G. F. Marrian. In this way, both brevity and association with the original name given to the pigment are retained, and at the same time chemical significance is added to the names for carotenoid alcohols by the ending "–ol". Further standardization seems desirable for ketones such as capxanthin and rhodoxanthin, and for carotenoid acids, esters and ethers.

[A]



Beta-Carotene ($C_{40}H_{56}$)
Beta structure

[B]



Luteol (a carotenol[2]) ($C_{40}H_{56}O_2$)
Alpha structure

Another structure involves a shift of double bonds along the entire length of the conjugated system, causing conjugation to extend through carbons 5, 4, and 3, and 5′, 4′, and 3′, with keto groups on carbons 3 and 3′ and an additional double bond in the aliphatic chain. Rhodoxanthin results. In capsanthin, ring I is broken between carbons 5 and 6, with formation of a keto group on carbon 6, and a hydroxyl group on both 3 and 3′. In the carotenoid acids, carboxyl groups replace one or both of the terminal rings I and II at the ends of the conjugated chain. In some cases the chain length is decreased. In *gamma*-carotene, the ring II of [A] is broken between 1′ and 6′, with a double bond between 1′ and 2′.

For a complete summary of available chemical data, the reader is referred to reviews by Strain (290), Zechmeister (143, 316) and Bogert (19). Kuhn, Winterstein and Lederer (159) reviewed the properties of carotenols in 1931. Strain (290) has contributed extensive data on a large number of carotenoids, particularly the carotenols, and has summarized the melting points, optical rotations, number of double bonds and hydroxyl groups, and position of absorption maxima for 13 carotenes, 30 carotenols, 3 carotenol esters, and 7 carotenoid acids. No research worker in carotenol chemistry can afford to be without this monograph by Strain.

The occurrence and properties of *gamma*-carotene were discussed by Kuhn and Brockmann (150) and Winterstein (312). Mackinney (168) obtained good yields of this pigment from marsh dodder.

Matlack and Sando (177) isolated lycopene from American and Italian varieties of tomatoes and found these sources to contain identical lycopene pigments, as shown by absorption spectra and chemical analysis. Matlack (176) isolated *beta*-carotene and lycopene from grapefruit flesh.

Kuhn and co-workers have described the isolation and properties of taraxanthol (155), violaxanthol (158), rhodoxanthin (151), cryptoxanthol (153) and rubixanthol (154).

In a series of papers (126, 127, 128) on the carotenoids of the purple bacteria, Karrer and Solmssen presented absorption maxima, melting points, and adsorption properties of three new pigments: rhodoviolascin, rhodopin and rhodopurpurin. Rhodoviolascin was shown to have both rings open, each with a methoxy group on carbons 3 and 3′; ring I has three double bonds which are conjugated with those of the chain.

Since they all have the same type of highly unsaturated structure, carotenoids are very easily oxidized by oxygen and stronger oxidizing agents. They must be kept in vacuum or under an inert atmosphere such as pure nitrogen. Carotenoids are stable toward alkali but very sensitive to acid and traces of acid must be strictly avoided.

Adsorption Characteristics

The method that has been most fruitful in the purification of the carotenoids and chlorophylls is that originally used by the botanist Tswett (296) to demonstrate the existence of numerous pigments in plant extracts by differential adsorption on the surface of a powder. In recent years this method has been applied to purification procedures for many natural pigments and its use has led to both success and disappointment. Each application must be studied in detail to avoid spurious results. Wilson (309) presented a mathematical treatment of a theory of chromatography, illustrated by various examples. In this section, application of the adsorption method will be discussed only as applied to carotenoids.

Essentially, the adsorption method consists of pouring a solution of pigments over a tightly packed column of powder in a glass tube, to the bottom of which suction is applied. The tube is constricted near the bottom to prevent movement of the powder. As the solution penetrates the powder, the pigments are differentially adsorbed while the solvent filters through into the suction flask. Since molecules having different structures are adsorbed on a given powder from a given solvent to different degrees, this process leads to a separation of the pigments into different zones on the adsorbent. After all of the pigments are adsorbed on the powder, the column is washed with pure solvent to promote a more quantitative separation of the zones. Under certain conditions, one zone may separate entirely from the one above it, leaving a colorless space between zones. More often the zones must be separated mechanically after pushing the column of wet powder out of the tube from the bottom or by scraping from the top.

The success of this method for any particular separation depends upon a great many factors, among which are the following: nature and source of the adsorbent, previous treatment of the adsorbent (especially with respect to drying and exposure to acid or alkali), texture of powder, packing qualities, method of packing, nature of

solvent, temperature, presence of oxygen, *etc.* Interfering substances may be adsorbed more strongly than the pigments, or so dilute the adsorbed pigments that color differences will not be apparent. Fraps, Kemmerer and Greenberg (68) studied the adsorptive power for carotene of many samples of magnesium compounds, including the oxide, carbonate and hydroxide. It varied greatly with heat treatment, and exposure to air, carbon dioxide or water. They have developed specific adsorbents (69) for the adsorption of (*a*) carotenol but not lycopene or carotene and (*b*) lycopene but not carotene.

Strain (290) has obtained good results with a special magnesium oxide powder (287) as adsorbing agent. This is mixed with 50% Super Cel to obtain good mechanical and filtration properties. Typical location of pigment zones (290) is as follows, from column top to bottom: carotenols, lycopene, *gamma*-carotene, *beta*-carotene, and *alpha*-carotene. Carotenols would be adsorbed in the following order: neoxanthin, zeaxanthol, luteol, and cryptoxanthol. Calcium oxide has been used by Miller (188) and alumina by Kuhn (150) for carotene separation. The carotenols have been separated with both calcium carbonate and magnesia.

Adsorption is thus stronger for the carotenols than for the hydrocarbon carotenes and increases with the number of hydroxyl groups present. Among the carotenes, strength of adsorption decreases with decrease in number of double bonds and with decrease in the length of the system of conjugated double bonds. Esters are ordinarily adsorbed in an intermediate position between carotenols and carotenes.

Several detailed discussions of the adsorption method have been presented in Zechmeister and Cholnoky's "Adsorptionsmethode" (317) and in Strain's "Leaf Xanthophylls" (290). Colored reproductions of carotenoid columns are presented in the frontispiece of Strain's monograph (290).

Absorption Spectra

In spite of instrumental differences remaining between spectrophotometers of different laboratories, the workers of the United States are now in fair agreement [see Smith (252) and Miller (189)] as to the absolute values of absorption coefficients in the visible region, as well as to wavelengths of maximum absorption, of the more common carotenoid solutions. This applies particularly

to those laboratories which employ the spectro-photoelectric method. Rather large differences (*i.e.*, 17–30%) in absolute values remain when certain European work, particularly that of Kuhn (149) and Smakula (248), is compared with American data. No complete explanation for these differences has appeared. Smith (252) presented a critical comparison of the absorption maxima for *alpha* and *beta* carotenes, as observed by the spectroscope, spectrophotometer and photoelectric spectrophotometer in the visible region, and points out how one may report erroneous wavelengths of maxima because of failure to consider certain factors that influence visual observations. The use of filters in making visual observations of absorption spectra with a spectroscope may lead to apparent shifts in the location of maxima. Kuhn (149) had previously called attention to an optic-physiological reason for differences between spectra observed visually and photoelectrically. Zechmeister (318, 319, 321) described the effect of age of solutions on their absorption spectra.

Absorption spectra of three representative carotenoids, determined by the spectro-photoelectric method, are presented in Fig. 1

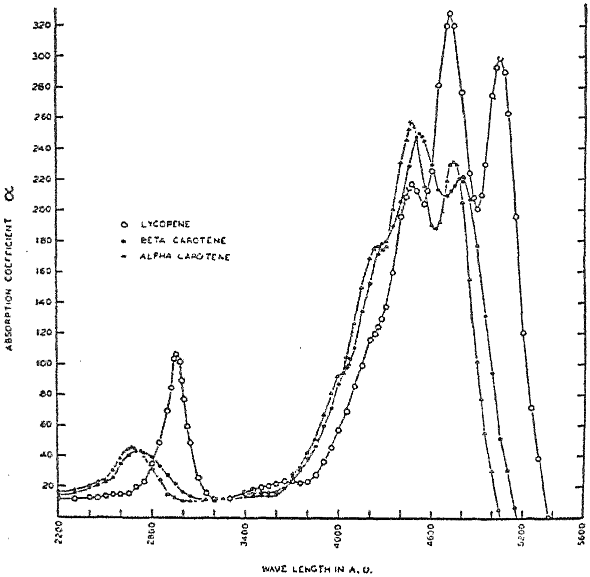


FIG. 1. Absorption spectra of *alpha* and *beta* carotenes and lycopene in 20% di-ethyl ether-80% ethanol [from Miller (189)]. [Reprinted from Plant Physiology.]

(189). The curves for *beta* and *alpha* carotenes illustrate the spectral differences due to the two positions of the double bond in ring II of the molecular structures presented above. This is a displacement of one double bond from a series of 11 conjugated bonds. It is to be noted that the different structure of the lycopene molecule accounts for a comparatively large change in both intensity and wavelength of the absorption maxima. This is particularly prominent in the ultraviolet region. Lycopene has one of the highest molecular absorption coefficients of all organic compounds whose absorption spectra in solution have been accurately measured. The carotenoids as a class absorb very strongly at their absorption maxima.

Heilbron *et al.* (13, 102, 103) presented absorption spectra of numerous compounds of the polyene series, which are derivatives of carotenoids.

Kar (124) presented absorption data for *beta*-carotene that are in good agreement with those of Miller, Mackinney and Zscheile (194) and Rudolph (222). Data for luteol solutions in ether were also given. After saponification to remove chlorophyll, Kar analyzed for total carotenoids by use of these data.

Gillam (83) presented ultraviolet and visible absorption spectra of the following carotenoids in chloroform solution: luteol, lycopene, zeaxanthol, violaxanthol, fucoxanthin, azafrin and astacene. Observations were made spectrographically and with a visual spectrophotometer on samples supplied by Kuhn.

Kuhn and Smakula (157) presented absorption curves for luteol and zeaxanthol in carbon disulfide solution, measured spectrophotoelectrically. Strain (289) reported considerable absorption by luteol and *beta*-carotene in the red region where chlorophyll exhibits maximum absorption.

McNicholas (179) followed the visible and ultraviolet absorption spectra of carotene and carotenol in solution during slow oxidation in the light. Visual and photographic methods were employed. Oxidation caused a decrease in the visible absorption and an increase in ultraviolet absorption. Two stages of oxidation were distinguished.

Karrer and Straus (129) used absorption coefficients to study the stability of colloidal solutions of *alpha* and *beta* carotenes in water. Absorption decreased on standing, especially in the light

and at elevated temperatures. Carotene combines with albumin to form a water-soluble solution. The properties of certain protein and lipoid systems containing carotene were studied.

It is well known that the absorption bands of solutions tend to become more narrow when measured at low temperatures. The spectra of *alpha* and *beta* carotenes at − 195° C. were measured with the spectrographic method by Miller (185). Similar measurements on *beta*-carotene, lycopene, leaf carotenol and other derivatives were made by Hilbert and Jansen (108). The wavelengths of maxima reported by these workers for *alpha*-carotene do not agree with those of Miller. Bowden and Morris (20) photographed the spectra of *beta*-carotene, vitamin A, and of an irradiation product of *beta*-carotene in ethanol at liquid air temperature. Microphotometer tracings of the spectra were given. Only wavelengths were measured in these cases, since accurate concentrations and cell thickness could not be determined for the glasses resulting from the frozen solutions.

Recently, Lewis and Calvin published a very excellent exposition on "The Color of Organic Substances" (161). A section is devoted to nearly linear molecules, and the carotenoids are considered here with other more simple polyene compounds. Although polyenes have a zigzag arrangement of carbon atoms in the chain, these writers regard them as linear for color considerations. After reviewing the experimental work of Hausser, Kuhn and Seitz (99) and of Smakula (248), absorption spectra of a series of diphenyl polyenes and carotenoids at room temperature and at − 196° C., and after making certain simplifying assumptions regarding unit oscillators and dipole moments within the molecule, Lewis and Calvin derived an equation:

$$\lambda^2 = k'n$$

in which λ is the wavelength of the absorption band of lowest frequency (highest wavelength), k' is a derived proportionality constant and n is the number of double bond units in the polyenic chain. The data of Hausser, Kuhn and Seitz (99) at − 196° C., plotted λ^2 *vs.* n, lie on a straight line, verifying the above equation. Values of n from 1 to 7 are included. Intensities of the first bands are also a linear function of n.

The absorption curves for numerous carotenoids presented by Smakula (248) all show an ultraviolet band, the wavelength of

which bears a strikingly constant ratio to that of the band of lowest frequency. Lewis and Calvin assign these first and second order bands to the fundamental oscillation of the molecule as a whole, rather than to certain parts of the molecule. For further information on the application of resonance theories to such molecules, the reader should consult the above paper by Lewis and Calvin (161) and various publications of Pauling (207, 208). Mulliken (197, 198) discussed theoretical aspects of the spectra of certain carotenoids and related compounds in relation to molecular shapes and details of atomic arrangements within the molecule.

Analytical Methods

Most analytical methods for determination of pigment content depend upon a separation of pigments, followed by the separate estimation of each. Often groups of pigments are thus separated from the extract and no attempt is made to determine individual pigments within the group. All of these pigments are extremely sensitive to acid, oxidation and heat; therefore, any reliable procedure must be as short and simple as possible, in order to avoid these causes of decomposition and possible enzyme action. The purposes of a pigment analysis must of course be borne in mind when an analytical procedure is planned. For instance, the determination of total carotenoids would not require the same method that would be necessary to determine the various carotenoid isomers individually. An estimation of vitamin A activity would not require the same type of analysis which would be in order if small quantities of certain carotenoid isomers were to be studied from the standpoint of plant physiology or genetics. These purposes and the equipment available are probably the two chief factors that will influence the choice of method from the many available at the present time. Other factors which enter into method consideration are: sensitivity, amount of plant material required, time, and universality of application (wavelength region, type of substance, *etc.*). In the following paragraphs, several methods will be briefly discussed and criticized.

Partition between immiscible solvents. When a solution of pigments is washed with an immiscible solvent, the pigments will become distributed between the solvents in a definite ratio, depending upon the chemical characteristics of solute in relation to those of the

solvents employed. For example, in a system of petroleum ether and aqueous methyl alcohol, carotenes will predominate in the upper phase, while carotenols will be more abundant in the lower alcoholic phase, since each compound seeks a solvent resembling itself in chemical nature. This general method has been used with various modifications by Willstätter and Stoll (310), Schertz (227, 228), Peterson, Hughes and Payne (213), Guilbert (91), Buxton and Dombrow (26), Buxton (25), and others. Zimmerman, Tressler and Maynard (322) employed diacetone alcohol to extract the pigments from fresh plant material. After addition of petroleum ether, the separation of pigment groups is controlled by the water content of the diacetone layer. The carotenes (in the petroleum ether) may be separated from the carotenols and chlorophylls in the lower aqueous diacetone layer. Chlorophylls may be saponified and removed from ether solutions of carotenoids by an aqueous layer. The method of chlorophyll analysis employed by Willstätter and Stoll (310) depends upon a partition between hydrochloric acid solution and ether. The concentration of the individual solutions may be estimated by a suitable colorimeter.

In many cases, entirely too much faith has been placed in this method from the quantitative standpoint, partly because the colorimetric method employed is incapable of distinguishing spectral differences in solutions of different composition, and partly because such separations between immiscible solvents are seldom quantitative, even after repeated washing of both upper and lower phases. This is particularly true of biological mixtures. The presence of different proportions of individual pigments and colorless impurities may change the solubility relations for a given pigment so that it does not distribute itself between solvents in the same ratio that would obtain if the pigments were pure. This is very noticeable when the method is used for isolation of pigments, and throws considerable doubt upon such analytical results from plant extracts. For instance, in the Willstätter and Stoll procedure (311) for separation of chlorophylls *a* and *b*, the results are influenced by the relative amounts of carotenoids present. In isolation work, however, the method has been very useful from the qualitative standpoint.

Adsorption analysis. By the use of small adsorption columns (1 mm. diam.) and correspondingly small amounts of solution, Strain (290) has separated as little as 0.0015 mg. of carotene into

the *alpha* and *beta* isomers. If the identity of such fractions is unknown, they may be mixed with known samples of pure pigment and readsorbed on a suitable column. If a single homogeneous zone appears, it may be concluded that the two pigments are very much alike or identical. If two zones appear, the pigments are certainly different. This method is very useful for identification purposes, as well as for the detection of trace amounts.

Sherman (242) applied this method to the identification and evaluation of carotenes from mature soybeans.

Fox (65) studied carotenoid pigments found in deep marine muds. Chromatographic and spectroscopic studies indicated that unesterified carotenols preponderate over carotenes and esterified carotenols. *Alpha* and *beta* carotenes and luteol were isolated from marine mud.

Strott (291) employed the adsorption technique to separate chlorophylls *a* and *b,* carotene and carotenol fractions before estimation with a visual photometer. One-half gram samples of fresh leaves were analyzed in a study of pigment development in etiolated plants and of pigment content of plants grown under different colors of light. Other physiological studies were also made.

Visual colorimetry. Many standards have been used for colorimetric comparison of pigment solutions. Examples of visual filter photometers are referred to below (6, 50).

The following workers employed potassium dichromate as a standard in a visual colorimetric method: Fraps, Treichler and Kemmerer (71) determined carotene content of various hays and corn; Sjøberg (247) estimated carotene and carotenols in plants during development; O. Smith (255) studied the effect of light upon carotenoid formation in tomato fruits, lycopene not being distinguished from carotene; Russell, Taylor and Chichester (223) analyzed alfalfa for carotenoids.

Clausen and McCoord (32) determined carotene and carotenol in blood plasma and serum by colorimetric measurements on solutions resulting from distribution between phases of hexane and diacetone alcohol. Solutions of potassium dichromate were standardized against samples of carotene and leaf carotenol.

Shrewsbury and Kraybill (244) found that carotene dissolved in butterfat shows a color intensity higher than when dissolved in petroleum ether. This is in agreement with findings of Goldblatt

and Barnett (90). Shrewsbury and Kraybill found the use of potassium dichromate unsatisfactory as a standard in analysis of melted butter and obtained more reliable results with colorimetric and visual spectro-photometric methods applied to petroleum ether solutions.

Kuhn and Brockmann (152) employed a micro-colorimetric method for the estimation of carotenoids, with azobenzene as a standard. Separations were first made by adsorption, phase separations, and saponification. Colorimetric equivalents and absorption bands were determined for *alpha* and *beta* carotenes, luteol, zeaxanthol, taraxanthol, violaxanthol, helenien, and physalien. They applied the method to numerous natural pigment sources. Dániel and Scheff (37) applied this method to blood carotene.

Watkins (306) employed a dye mixture as a colorimetric standard with which to study the carotene content of range grasses.

Holmes and Bromund (111) proposed the use of a bixin standard for the colorimetric determination of carotene in benzene and chloroform solutions.

Employing *beta*-carotene as a standard, Bruins, Overhoff, and Wolff (23) determined carotene with a visual colorimeter.

Ferguson and Bishop (57) used a tintometer, in which colors of extracts were compared with standard yellow glasses. Methods were given for various agricultural products.

Sumner and Fox (292) determined colorimetrically the carotenol content of fishes grown under controlled color environment.

Munsey (199) discussed in detail the application of the neutral wedge photometer to carotenoid determination, with particular reference to flour products.

Quackenbush, Steenbock, and Peterson (219) studied the effect of acid on carotenoids, especially in relation to alfalfa silage. It was pointed out that the usual methods of carotene analysis fail to differentiate between carotene and certain derivatives which are formed by acid and which possess no vitamin A activity.

In these visual colorimetric methods, the color intensities of the unknown and standard solutions are compared visually and concentrations are determined from calibration curves. These methods are comparatively simple and rapid but subject to severe limitations. Since chlorophyll and carotenoids are not stable in solution and since tests of purity are not extremely simple, it is necessary to prepare a fresh and purified sample of pigment to use

as a standard for each series of unknowns; this is impractical for routine work. Mixtures of pigments commercially available cannot be depended upon to be 100% pure. For instance, when mixtures of *alpha* and *beta* carotenes are used as standards, the percentage composition of the standard is not reproducible and often not known. Variations in carotenoid composition may cause slight changes in color and accurate matching of intensities of unequal colors is visually impossible and physically difficult to translate into concentrations. This difficulty often arises with use of a stable secondary standard which is calibrated against pure pigments.

Visual spectrophotometry. Buxton (25) employed a visual spectrophotometer to determine absorption values at 4500 Å on mixtures of carotene and cryptoxanthol from yellow corn. Cryptoxanthol was then adsorbed on calcium carbonate and carotene determined on the filtrate. The difference was taken as a measure of cryptoxanthol. He was unable to distinguish between them spectro-photometrically when they occurred in the same solution. Buxton and Dombrow (26) used the visual spectrophotometer for study of the carotene content of alfalfa meal.

Sherman and Salmon (243) determined the carotene content of soybeans and cowpeas with a visual spectrophotometer and a *beta*-carotene standard.

Ferrari (58) described a spectro-photometric method, improved over that of Ferrari and Bailey (59, 60), for determination of carotenoids in flours. A visual spectrophotometer was used, with the 4358 Å line from a mercury arc as a source of light. Wiseman, Kane, Shinn, and Cary (315) employed the same method to determine carotene content of market hays and corn silage. They also used a photoelectric photometer (21) and a colorimeter with a potassium dichromate standard. With suitable calibration, all three methods gave similar results in their hands. See also a paper by Goldblatt and Barnett (90). Barnett (12) employed a color analyzer (142) to determine carotene in butter fat.

Peterson, Hughes, and Freeman (212) reported absorption coefficients for *beta*-carotene in three solvents, as measured with a visual spectrophotometer. They modified Guilbert's method (91) for application to forage crops. The method of Petering, Wolman, and Hibbard (209) and modifications have been compared with the method of Peterson, Hughes, and Freeman (212) when it was

desired to determine carotene only (15). The necessity for further improvement was indicated.

Several types of visual spectrophotometers are manufactured, chiefly by Bausch and Lomb Optical Co., and the Gaertner Scientific Corporation. The Nutting Colorimeter (1) has been widely used.

Spectrography. This method has not proven highly successful, probably because of the difficulty of accurate determination of small differences in absorption from photographic plates. Only by the use of great caution and many correction factors can the error in spectrography be kept as low as 3%.

This method was used by Clark and Gring (31), who estimated the carotene and carotenol fractions of corn, and by Bills and McDonald (17), who studied the carotene content of ten carrot varieties.

Photoelectric filter photometry. It should be emphasized that certain more recent types (2, 7, 8, 29, 30, 63, 78, 165, 214, 215, 216, 308) of photoelectric filter photometers have reduced the errors of precision inherent in visual color comparison and are capable of giving very satisfactory results in cases where mixtures are not too complex and need not be resolved. For instance, since the filtered radiation employed is a relatively broad band (about 1000 Å) extending from 4000 to 5000 Å for total carotene determination, variations in *alpha* and *beta*-carotene composition do not interfere greatly with reliability of results on analysis for total carotene, if very small differences are not important. The carotene isomers cannot be distinguished by this method (245). The above list of references is fragmentary and many other types of equipment designed for similar analyses are available. More recently several relatively inexpensive types of grating instruments (28, 34) have become commercially available in this field.

Instrumental factors which affect the calibration curve of these instruments are: spectral distribution of radiation from the source, spectral transmission characteristics of all filters in the light path, and the spectral sensitivity curve for the photocells. A change in any of these factors may seriously alter the calibration curve. Several factors which the prospective purchaser should investigate carefully in photoelectric equipment are: (1) Spectral range and purity of radiation isolated from the source for the measurement (scattered light is very objectionable); (2) wavelength range of

instrument; (3) sensitivity; (4) precision of results; (5) type of photocell used (high sensitivity and absence of fatigue are desirable; (6) quality of optical parts; (7) general workmanship, including electrical connections and mechanical stability; and (8) temperature effects on sensitivity. Mellon (181) and Müller (196) have written excellent review articles on topics pertaining to these subjects.

Shrewsbury, Kraybill and Withrow (245) showed that a photoelectric photometer (308) permits determination of *alpha* or *beta*-carotene with an accuracy of 1%, with greater precision than is possible with a visual spectrophotometer.

Koehn and Sherman (147) employed a photoelectric colorimeter to determine *beta*-carotene. A visual spectrophotometer was used to standardize the photoelectric instrument.

Randolph and Hand (221) employed a photoelectric colorimeter (95) with a calibration curve obtained from pure *beta*-carotene, to study the relation between total carotenoid content and number of genes per cell in diploid and tetraploid corn. Great variations in carotenoid content between commercial varieties were found and these were definitely related to chromosome content.

A photoelectric photometer was used by Zimmermann, Tressler and Maynard (322) to determine carotene content of vegetables, by Hodgson (110) to study the carotene content of dairy feeds, and by Brooke, Tyler and Baker (22) to determine carotene in alfalfa meal.

Fraps *et al.* (70) employed a rapid adsorption method for the determination of carotene. Impurities were adsorbed and the carotene remaining in solution was determined with a photoelectric colorimeter.

The Molisch reaction, which produces a distinct color change when sulfuric acid is added to a carotenoid solution, has been only qualitatively useful because the color produced is transient and its intensity is probably dependent on concentration of acid. The Carr-Price color reaction with $SbCl_3$ likewise has not been quantitatively reliable.

Photoelectric spectrophotometry. This method was first applied to carotenoid solutions by Miller (186, 187) and has been employed by him to include three and four components (190), namely: mixtures of *beta*-carotene, lycopene, and "leaf xanthophyll"; and of *beta*-carotene, "leaf xanthophyll," chlorophyll *a*,

and chlorophyll *b*. Johnson and Miller (118, 119) used this method of analysis to study the variation in carotenoid pigment concentration among cross-bred strains of corn and in several grasses (120).

This method has a greater number of advantages than any of the other methods. The time consumed in the observation is a matter of only several minutes for each determination and the total amount of material involved is approximately .03 mg. in 10 cc., a concentration of 3 mg. per liter. It is therefore a truly micro method. Moreover, the sensitivity is very high when it is considered that the individual pigments need not be separated from each other for analysis and that a content of several per cent of a single pigment of the *beta* configuration can be detected in the presence of another pigment of the *alpha* configuration in a sample of .03 mg. *total* pigment. High accuracy in this respect has been attained in most cases only for mixtures of the purified pigment standards. This ability to distinguish between and analyze for pigments having spectra as close as those of *alpha* and *beta*-carotene is dependent upon the use of highly monochromatized radiation.

The method has several disadvantages. Rather expensive optical apparatus is required and the fundamental absorption data must be obtained from pure samples of each pigment to be included in the analytical system. This necessitates an initial preparation period before analytical results may be forthcoming from such an instrument. Moreover, the spectra of all pigments present must be known before analytical data upon unknowns can be interpreted accurately. Assumption of the presence of certain pigments leads either to a successful analysis, in which case the assumption must have been correct, or to failure, in which case the assumption is proven wrong and either (*a*) another pigment must be identified and studied, (*b*) spectral changes have occurred during the extraction process, or (*c*) the standards do not have the spectral properties of the pigments in the extract. The writer knows of five laboratories in the United States which have this type of equipment. Sufficient data on standard solutions should be available soon so that inter-laboratory comparisons can be made easily and accurately. Then it should not be necessary for every laboratory to prepare every pigment for its own fundamental data, but such data obtained once will be useful in other places. This should be true,

at least among laboratories employing the same kind of optical equipment under the same conditions and thereby employing the same purity of radiation. Close cooperation between laboratories in the establishment of standards and in the complete description of experimental conditions in force when fundamental absorption constants are obtained is necessary for a wide and full application of the method to pigment spectroscopy. Unpublished data from the writer's laboratory indicate at present that successful application of this method with high accuracy to plant extracts is not as simple and straight-forward as might be inferred from simple experiments with artificially-made mixtures of pigments. This is partially a result of the high sensitivity of the method. It is, however, comparatively easy for this method to surpass others in both sensitivity and accuracy. The high sensitivity increases the possibilities for improvement of methods, and for identification and study of new pigments.

In all optical methods, the greatest care should be taken in regard to the proper use of absorption coefficients or transmission values of pigment standards. These may vary considerably, depending upon the quality of radiation employed in the determination. The effect of various slit conditions and scattered radiation has been too often neglected. For this reason it is not justifiable to employ standard values obtained with one method or instrument in another without an accurate knowledge of the physical factors involved.

Miscellaneous methods. Connor (36) attempted, with little success, to differentiate carotene and carotenols from each other in tissues by staining technique.

Analysis by the measurement of the optical rotatory power of carotenoid solutions has been reported by Kuhn (149). Such an analysis can be only partial, however.

Fluorescence Spectra

Hausser *et al.* (99, 100) measured fluorescence spectra of various diphenylpolyenes, both at room temperature and at − 190° C. Fluorescence was excited by mercury arc radiation. The carotenoids so far examined have three or four fluorescence bands (45).

Dhéré (45) discussed the fluorescence of vitamin A, carotene and lycopene solutions at − 180° C. These spectra consist of several distinct bands in the yellowish-green region of the spectrum.

General Physical Properties

Melting points of carotenoids, as usually determined in an open melting point tube with a thermometer, have not been satisfactory physical constants because of decomposition with melting and progressive oxidation during the heating period. Values are usually reported over a range of one or more degrees. Moreover, many carotenoids melt within a few degrees of each other and small amounts of impurities cause confusion in identification by this method. Since many carotenoids crystallize with from 1 to 25% of solvent of crystallization, Strain (290) has employed the practice of drying these pigments in a vacuum at 84° C. in an all-glass apparatus. Recently, Zscheile and White (330) employed a microscope hot stage to determine melting points of individual carotenoid crystals with a precision of $\pm$ 0.03° C. between determinations on the same sample. The crystals are heated at a known uniform rate in an atmosphere of purified nitrogen, and although decomposition still occurs, oxidation is avoided and the melting point is observed at a very sharply defined temperature. Observation of the melting point is facilitated by the use of polarized light.

The physical appearance, color, and crystalline form of carotenoid crystals may vary considerably with the solvent employed, the rate of crystal growth, relative dryness, solvent of crystallization, method of illumination, purity, and nature of contaminants. This renders microscopic identification difficult and uncertain.

Mackinney (167) and Mackinney and Milner (174) compared the melting points, optical activities, and absorption maxima (visual) of carotenes from many plant species. *Beta*-carotene predominated in all cases. Strain (288) studied carotenes from many different plant sources. Melting points, optical rotations, absorption maxima, and specific gravities for *alpha* and *beta* carotenes were determined. The optical rotation of *alpha*-carotene was measured in four solvents for the 6678 Å helium line. The specific gravity for both carotenes was 1.000 $\pm$ .004 referred to water at 20° C.

Smith (250) studied the absorption spectra, density, refractive index, and optical rotation of carotene and certain hydrogenated derivatives. Smith and Milner (253) measured the optical rotations, melting points, solubilities, and absorption spectra of carotenes from many plant sources. The absorption spectra were

photographed and a microphotometric record of the plates was made.

Staudinger and Steinhofer (262) measured the viscosities of solutions of *beta*-carotene, lycopene and other carotenoids in certain solvents. These were correlated with the number of double bonds per molecule.

Schertz measured the solubility and studied the stability of carotene (229) and carotenol (230) under several conditions.

Mackinney (166) studied crystals of carotene, luteol, and lycopene by the x-ray diffraction method and determined interplanar spacings. The powders were exposed in vacuum. He found definite differences, both of spacing and intensity, and suggestions of common structural features. A new unit cell for *beta*-carotene has been proposed by Taylor (293) and discussed by Mackinney (173). A complete interpretation of this type of data is lacking.

Since carotenoids have a large number of double bonds, many cis-trans isomers are theoretically possible. *Alpha*-carotene and luteol are dextrorotatory, due to the asymmetry of carbon 6′ (formula [B]). *Beta*-carotene and cryptoxanthol are optically inactive. Isomerization has caused considerable confusion in the purification and characterization of certain carotenoids.

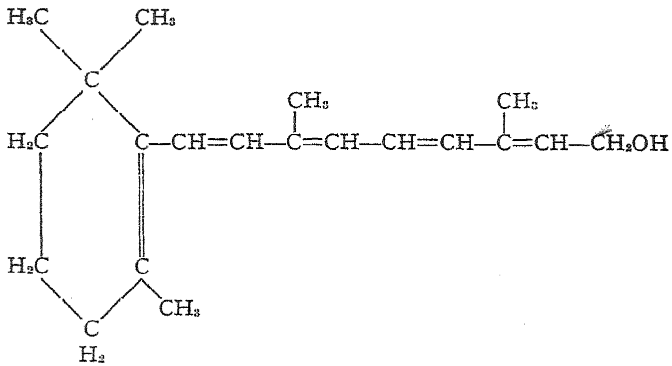
Kuhn (149) used repeated crystallization with iodine to purify *alpha*-carotene. When *beta*-carotene is precipitated by iodine, an isomerization occurs and *beta*-carotene cannot be regenerated unchanged by addition of mercury. Iso-carotene results which is optically active and exhibits no vitamin A activity.

Gillam *et al.* (85) determined the ultraviolet and visible absorption spectra of carotene before and after isomerization with antimony trichloride. This type of isomerization caused a shift of absorption maxima to longer wavelengths. The carotenoids may isomerize under certain conditions, such as high temperatures in solution, to other products, probably geometrical isomers. Gillam *et al.* (86, 87, 89) at first considered adsorption to be the cause of isomerization but later Carter and Gillam (26*a*) agreed with Zechmeister (320) that an equilibrium exists in such cases and that isomerization to the neo-products occurs least rapidly in the solid state in the cold. The carotenols are more susceptible to isomerization than the carotenes. The type of isomerization which results from heat and standing in solution causes a shift in the absorption maxima toward the shorter wavelengths (320).

Vitamin A Activity

To date the great majority of carotenoid analyses have been made because of an interest in the vitamin A activity possessed by certain carotenoids. The brilliant chemical work of Kuhn, Karrer, and others (see 213) has shown that of all the carotenoids commonly found in nature, only four possess vitamin A activity. In addition, Scheunert and Wagner (234) have reported very recently that aphanin is one half and aphanicin one fourth as potent as *beta*-carotene in vitamin A activity as shown by animal assay methods. It is evident from formula [A] that *beta*-carotene might be hydrolyzed by a rupture of double bonds between carbons 15 and 15′ (formula [A]) to form two molecules of the alcohol [C], which is now known as vitamin A_1. The unbroken *beta*-ionone ring is essential for the biological activity of the resulting alcohol and therefore, when *alpha* and *gamma* carotenes are hydrolyzed, only one vitamin A_1 molecule can be formed from each carotene molecule. The presence of a hydroxyl group on the ionone ring prevents vitamin activity. Thus cryptoxanthol is the only carotenol so far shown to exhibit vitamin A activity. Karrer (125) has reviewed the chemical relations and vitamin A activity of *alpha, beta* and *gamma* carotenes.

[C]



Vitamin A_1

Vitamin A was first described as an oil at room temperature, light yellow in color. It was purified from natural sources in 1937 by Hickman (106) by vacuum distillation and by Holmes and Corbet in 1937 by means of fractional crystallization (112). This oil had a broad absorption maximum at 3280 Å and a melting point of

7.5–8.0° C. In this same year, Kuhn and Morris (156) synthesized a product which gave the antimony trichloride reaction, and which showed the same chromatographic behavior and kind of biological activity as vitamin A_1. However, they did not isolate it in a highly purified state. The adsorption behavior of carotene and crude vitamin A on several adsorbents has been studied by Holmes *et al.* (112). Mead (180) found solvent of crystallization in vitamin A_1 prepared by Holmes and Corbet's method (112). For the solvent-free alcohol, he found 1800 as the $E^{1\%}_{1cm}$ value.

Baxter and Robeson (14) recently prepared pale yellow prismatic crystals of vitamin A_1 alcohol, which were solvent-free and melted at 63–64° C. They also synthesized vitamin A_1 palmitate from the alcohol. The palmitate crystals melted at 26–28° C. Propylene oxide was the solvent used for crystallization. These workers reported a maximum $E^{1\%}_{1cm}$ value of 1850 at 3280 Å and an average value of 1725. Considering data from both the alcohol and palmitate, they obtain 1720 as a reliable value. This is 4% lower than Mead's values. Perhaps variations in spectro-photometric techniques are responsible for these differences (14). Gillam and Ridi (88) studied the effect of solvent upon the absorption coefficients of vitamin A_1.

Gillam *et al.* (84) investigated vitamin A_2, a higher alcohol with one more double bond and correspondingly longer conjugated chain than vitamin A_1. Vitamin A_2 is found in livers of certain fresh water fish. Absorption maxima for this derivative are at 2850 and 3450 Å. Colorimetric methods for the differentiation of this vitamin from vitamin A_1 were studied by Lederer and Rathmann (160). Embree and Shantz (51) followed the cyclization of vitamin A_2 spectrographically and based an analytical method for the two vitamins on the different adsorption properties of their cyclized derivatives.

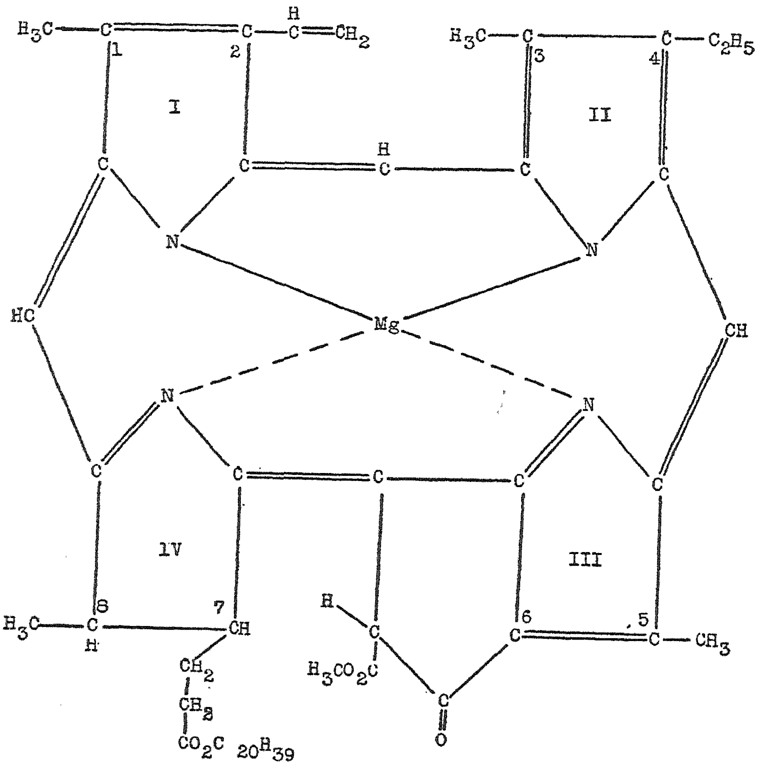
From the analytical viewpoint, analysis for vitamin A activity in plant tissues is very difficult because of the coexistence within the plant of so many pigments closely related to, and spectroscopically indistinguishable from, those which are precursors of vitamin A. Therefore, it is important to search for methods or spectroscopic regions which can be used to detect and measure smaller molecular differences. Some of the many pigments having no vitamin activity must be included in any spectroscopic analytical system simply because they are probably present in most biological extracts and

as yet cannot be quantitatively removed or preferentially separated from those which are precursors of vitamin A.

CHLOROPHYLLS

Chemical Relationships and Reactions

A structural formula of chlorophyll *a*, according to H. Fischer (61), follows:



Chlorophyll *a* ($C_{55}H_{72}O_5N_4Mg$)

In this revision of the structural formula, two hydrogen atoms have been moved from carbons 5 and 6 to 7 and 8. In chlorophyll *b*, the methyl ($-CH_3$) group on carbon 3 is replaced by a formyl $\left(-C\begin{smallmatrix}\nearrow O\\ \searrow H\end{smallmatrix}\right)$ group. The chemical properties of these two components of the chlorophyll complex have become fairly well estab-

lished. The structural chemistry of chlorophyll was reviewed by Steele (263).

Rabinowitch and Weiss described the reversible oxidation of alcoholic chlorophyll solutions by ferric ion (220). A reversible decrease in fluorescence intensity accompanies this oxidation. These changes were traced by photoelectric methods and spectral changes in absorption which occur during oxidation were studied. Rabinowitch's work and theories on the nature of these reactions suggest many interesting problems. Porret and Rabinowitch also described (217) a reversible bleaching of chlorophyll solutions by light and postulated a dissociation into dehydrochlorophyll and a hydrogen atom.

Blaydes (18) described a method for the preservation of the green color of plant specimens by addition of copper sulfate to the preservative solution. A stable compound between copper and chlorophyll results.

Adsorption Properties

During the last decade the earlier methods of chlorophyll preparation (233, 310) have been considerably modified in certain important details. The use of the Tswett adsorption technique has made it possible to obtain the chlorophyll components individually in a state of greater purity than was possible by the earlier method of Willstätter and Stoll (310), which depends upon partition between immiscible solvents and is incapable of effecting a quantitative separation of chlorophyll *a* from *b* because no solvents have been found in which the distribution coefficients for these pigments are sufficiently different. Talc had been used considerably as an adsorbent but it has since been shown that its quality is not readily reproducible and its use is to be discouraged (172, 314). Commercial powdered sucrose is inexpensive, easily accessible, and very satisfactory. Its solubility in water makes it very easy to eliminate from ether elutriates of chlorophyll. Inulin has been used by Mackinney (172), especially for the purification of component *b*. He found that it has the advantage of giving a discrete zone of the *b* component, separated from other zones. He has also used starch and magnesium citrate as adsorbents. The degree of dryness has been shown to be very important for the effectiveness of adsorbents (172).

The adsorption technique is only a part of the entire purification

procedure of chlorophyll. Certain essential steps of the Willstätter-Stoll method are retained in the methods of Mackinney (172), Winterstein and Stein (314), Zscheile (323), and Zscheile and Comar (328). The more important recent improvements have been in the choice of better adsorbents (314), improved working methods (172), quicker extraction methods, and development of a better knowledge of the conditions which minimize decomposition (328). These have resulted in a reduction of the total time required, more complete purification of the components from each other, and less contamination with decomposition products.

A colored reproduction of two columns containing adsorbed chlorophyll was presented by Zscheile (323).

Absorption Spectra

The absorption spectrum is by far the best criterion of purity for the chlorophyll components. Chlorophyll has no definite melting point or other easily determined physical constant. Elementary analysis of chlorophyll cannot detect the small but significant differences which result from various treatments of the pigment and which Zscheile and Comar (328) have shown to be readily detectable spectroscopically. By means of spectroscopic data the preparations of Willstätter and Stoll, Winterstein and Stein, Zscheile (323), Egle (48), Mackinney (172), and Zscheile and Comar (328), have all been shown to be successive improvements over previous preparations. Thus according to Winterstein (311), Willstätter and Stoll's *b* contained about 15% *a*. Stoll and Wiedemann (286) maintain that the method of Willstätter and Stoll leads in practice to spectroscopically pure components *a* and *b*. However, spectroscopic observations of other investigators (314, 323) do not support this contention. Albers and Knorr (3) reported higher absorption coefficients for chlorophyll preparations which had previously been adsorbed on sucrose than for those prepared by the original Willstätter and Stoll procedure. In the component *b* preparation of Winterstein and Stein (314), the band at 6140 Å indicates the presence of *a* (48, 323). The preparations of Zscheile (323) and Mackinney (172) as well as of others probably had contaminations of pheophytin which are thus far impossible to avoid when a solid product is formed (328). Limitations of space do not permit a detailed comparison of these various preparations.

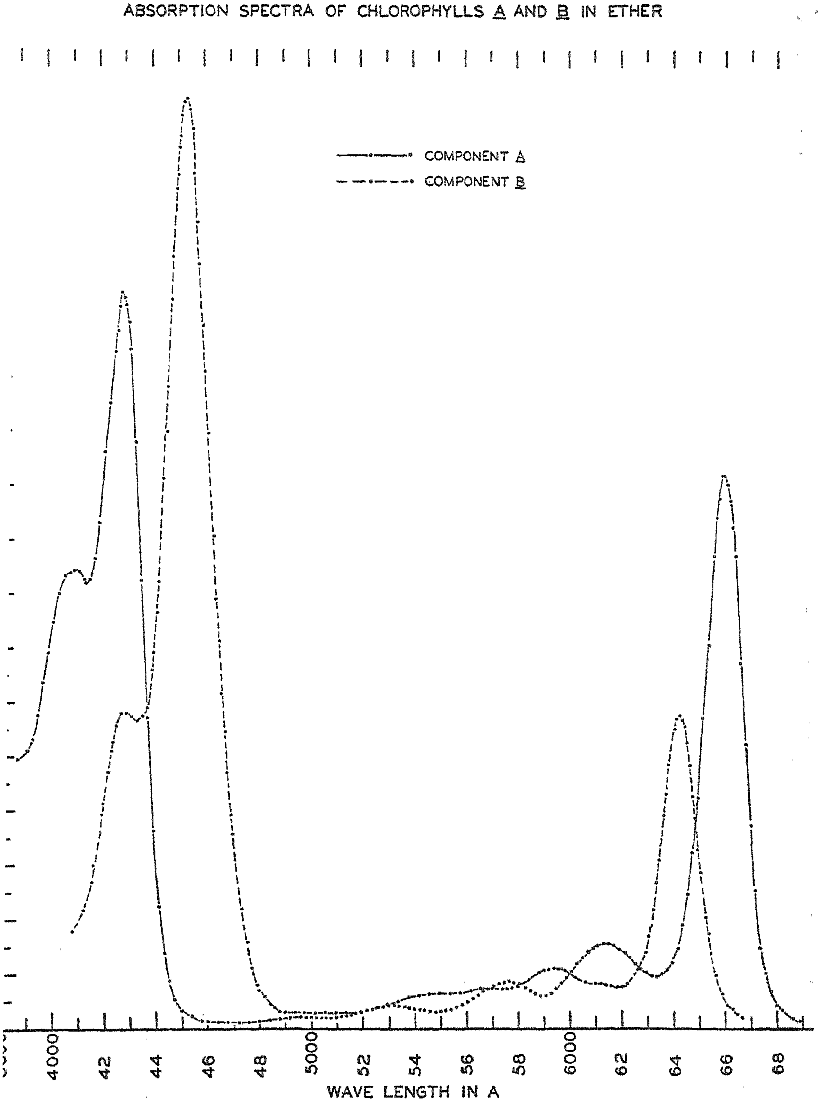


FIG. 2. Absorption spectra of chlorophylls *a* and *b* in ether solution [from Zscheile and Comar (328)].

Figure 2 shows the absorption spectra of chlorophyll *a* and *b* as obtained recently by Zscheile and Comar (328). When the highest absorption coefficient of chlorophyll *b* is converted to the molecular basis, its value is intermediate between the corresponding maximum values of lycopene and *beta*-carotene.

Mackinney (169) employed the spectro-photoelectric method to study relative absorption values, in the red region of the spectrum, of leaf extracts from leaves killed by various means. Later he presented (172) absorption spectra of chlorophylls *a* and *b* which had been rigorously purified by a simplified preparative procedure. Inulin and magnesium citrate were employed as adsorbents. His final products were dried over P_2O_5 in vacuo. Determinations of nitrogen and ash content were made on numerous preparations. Absorption spectra were studied in the visible range for acetone, benzene, and ether solutions and comparisons were made with results from other laboratories.

Rabinowitch and Weiss (220) studied the absorption spectra from 3500 to 7000 Å of Stoll's chlorophyll *a*, chlorophyll *b*, and the mixed ethyl chlorophyllides *a* and *b* in methanol solution. They employed a spectro-photoelectric method.

Biermacher (16, 46) further purified Stoll's component *b* by permitting particles of it to stand for long periods of time in hexane in the dark. The solvent was decanted frequently until fresh solvent became only slightly colored and gave no spectroscopic test for *a*. He claimed that in this way he removed *a* quantitatively from *b* and that the presence of the 6625 Å band in the fluorescence spectrum of *b* is the most sensitive test for the presence of *a*, more sensitive than one depending upon the 6600 Å absorption band of *a*.

Kar (124) found absorption coefficients for chlorophyllide *a* higher at 6600 Å and lower at 5500 Å than those determined by Rudolph (222) and Zscheile (323) for chlorophyll *a*. This comparison is not strictly valid because of difference in solvent and compound. Since the differences were small between the values of Kar and Zscheile, and because no *b* component was available to him, Kar accepted the absorption values of Zscheile for chlorophyll *b*.

Meyer (183) made extensive comparisons of the absorption spectra (spectrographic) of Stoll's chlorophyll *a* and *b*, a mixture of these, and of certain colloidal preparations and plant extracts. He stressed the importance of the region near 5300 Å as a means

of distinguishing between his so-called "native chlorophyll" and pure preparations. His colloidal preparation from "native chlorophyll", contrary to those previously reported, fluoresced and showed no Tyndall beam. The spectra of his colloidal preparations resembled those of leaves very closely.

Stern and co-workers (264 to 277, 282, 283) studied the visible absorption spectra of a large number of chlorophyll derivatives of the porphyrin class with a visual spectrophotometer. Most of this work was done with dioxan as solvent. They reported spectral differences for a very extensive list of derivatives formed by substitution of various groups within the molecule, including metallic substitutions.

Hagenbach *et al.* (94) studied the ultraviolet and visible absorption spectra of chlorophylls *a* and *b* and numerous related porphyrin pigments. A sector spectrophotometer and a visual spectrophotometer were employed.

Hubert (116) studied Beer's Law by use of filtered radiation applied to chlorophyll dissolved in several solvents. He found this law to be obeyed in his concentration range of 6.25–100 mg. per liter.

Lewkowitsch (162) examined the ultraviolet absorption spectrum of an alcoholic leaf extract spectrographically. These results are very qualitative.

Kundt's Rule, which relates the wavelength of absorption maxima with refractive index of solvent, has been studied by Hubert (115, 116), Katz and Wassink (130), Wakkie (300), Mackinney (170, 171), Baas Becking and Koning (9), and more extensively by Egle (48). Considerable disagreement exists between their results. The factors of sample purity and instrumental technique discussed above apply also to these studies.

Conant and Kamerling (35) photographed the visible absorption spectra of numerous porphyrins and chlorophyll derivatives at liquid air temperatures. The spectra of the former show groups of narrow bands while those of chlorophyll derivatives show wider bands. Changes caused by substituent groups were studied.

Padoa and Vita (206) photographed spectra of solutions of chlorophylls *a* and *b* in benzene solution saturated with various gases: oxygen, carbon dioxide, nitrogen, and carbon monoxide. They concluded that all of these gases, except nitrogen, form labile compounds with chlorophyll, causing a change in the absorption

Stair and Coblenz (161) employed prisms of fluorite and rock salt, with a thermopile and galvanometer system, to study the absorption spectra of films of pigments on a rock salt plate. Spectra of chlorophyll $(a+b)$, carotene, carotenol, ethyl chlorophyllide, phytol, pheophytin, and copper pheophytin were measured from 10,000 to 140,000 Å. Numerous bands were recorded in this region and no attempt was made to classify the maxima for theoretical considerations. The method of film preparation necessary for these measurements probably caused the film to be contaminated with much decomposed pigment, which makes interpretation difficult.

The molecular absorption coefficient of chlorophyll in the red region has been used by Kohn (148) to calculate the "functional" molecular absorption coefficient of chlorophyll.

Smith (249) has examined the absorption spectra of chlorophyll-protein compounds in solutions prepared from leaf extracts by the use of digitalin. Comparisons were made between such solutions and those of chlorophyll. Centrifugal studies indicated two different molecular weights, over 70,000, for these complexes with protein.

Albers and Knorr (4) photographed the absorption spectra in the red region of single chloroplasts of several species.

Katz and Wassink (130) prepared cell-free extracts from *Chlorella, Rhodospirillum rubrum,* and a strain of purple sulfur bacteria. Their absorption spectra in the red region resemble those of living cells very closely. Microscopic observation showed the presence of colloidal particles. Heat caused a new maximum to appear, which was interpreted as due to liberation of the pigment from the protein carrier. The use of different gas atmospheres did not influence absorption.

Mestre (33) considered in detail the many factors entering into the interpretation of measurements of radiation absorption by tissues, such as leaves and algae.

In 1934 Zscheile (323) presented inconclusive evidence that a third component "c" might exist in the chlorophyll complex of green leaves. The fraction which he isolated from adsorption columns of talc was between the layers containing chlorophylls *a* and *b* and its spectrum was somewhat intermediate between those of *a* and *b*. He was unable to purify it further by the technique then employed. Later (in 1936), on the basis of a single series of measurements (327), he obtained evidence which indicated that

this "*c*" fraction was a mixture of chlorophyll *a* and a degradation product of chlorophyll, probably produced in the preparative procedure. Recent (1940) results obtained by Zscheile and Comar indicate that no third component exists. This subject has been discussed at length by Winterstein and Schön (313), Mackinney (169, 172), and Bakker (10), none of whom found chlorophyll "*c*".

Various methods of absorption spectroscopy have been applied to chlorophyll and carotenoid solutions for many years. It has been very unfortunate that there has been no organized action among workers in this field to promote a uniform method of reporting this type of absorption data. The reader must be very careful to convert such data into corresponding units before comparisons are made.

Since many different types of equipment have been used to measure the absorption spectra of pigments in various laboratories, considerable confusion exists as to the numerical values of absorption coefficients. These values may vary when measured in different ways, because of purely instrumental differences. In addition to such differences are the inevitable possibilities of differences in sample purity. These may arise from isomerization, oxidation, action of acids, light, *etc.* Some workers make no attempt to determine absorption coefficients quantitatively, but are content to record only the wavelengths of maximum absorption. In such qualitative work, the minima of absorption are usually neglected and small but significant shoulders or points of inflection in the absorption curves are missed or unreported. Moreover, the base breadths of such curves are often totally neglected or determined with less accuracy than the maxima. These considerations will be of more significance when analytical methods for chlorophyll are discussed in the next section.

Analytical Methods

The general considerations presented above for the analytical methods for carotenoid pigments apply to many of the methods of chlorophyll analysis. Most methods are for total chlorophyll ($a + b$) and depend upon the absorption in the red region for their accuracy.

Zscheile and Comar (328) have shown that solid standards such as those of Stoll, Sandoz Ltd., *etc.* are not reliable. Miller (190)

and Miller and Johnson (193) have analyzed certain samples of tissue for chlorophyll, employing the spectro-photoelectric method. Their standards were the absorption spectra of chlorophylls *a* and *b* as determined by Zscheile (323, 324). However, since no account of the drying factor (328) was taken by either Zscheile or Miller, in the preparation of their standards, their values of absorption coefficients cannot have quantitatively accurate numerical significance when applied to plant extract analysis. The spectrophotoelectric method employed by Zscheile and Miller is capable of greater precision and accuracy than any others that have been applied to plant pigment analysis. It is emphasized that certain necessary and important factors of extraction and instrumental use must be recognized and a knowledge of them applied in detail in order to obtain reliable results.

It is the writer's opinion that none of the numerous chlorophyll analyses described in the literature can have sufficient accuracy to be more than very rough approximations for one or both of two reasons (*a*) pure standards were not available for instrument calibration (328) and (*b*) no account of *a* to *b* ratio variations could be taken in the method employed. Limitations of the physical technique employed have also been serious.

The more recently employed methods will be briefly mentioned here and some of the difficulties noted.

Visual colorimetry. Schertz (231) employed a visual colorimeter to estimate depth of color and a visual spectrometer to estimate band widths as compared to standard solutions of chlorophyllin salts $(a+b)$.

Sprague (257) and Sprague and Shive (259) modified the method of Schertz (231) to study pigment content of corn leaves. They used certain dye solutions as colorimetric standards, which in turn had been standardized against pigments supplied by Schertz. Later an improved dye mixture was devised as a color standard by Sprague and Troxler (260). Sprague and Curtis (258) then employed the chlorophyll $(a+b)$ content as an index of productive capacity of corn hybrids. Deuber (42) employed Schertz's method to study chlorophyll development in seedlings. He studied (43) the chlorophyll content of leaves from twenty tree species by the use of Guthrie's standard (92) and Schertz's (232) extraction method. Hicks and Panisset (107) employed a tintometer and a standard of

$a+b$ supplied by Schertz for the estimation of total chlorophyll in 80% methyl alcohol extracts.

Harriman (98) employed a colorimeter to study the effect of storage conditions on chlorophyll content of leaves. His standards were Schertz's chlorophyll and the inorganic standard of Guthrie (92).

Henrici (105) employed freshly saponified copper chlorophyll (supplied by Stoll) as a colorimetric standard for determination of chlorophyll in saponified extracts from grasses.

Guthrie (92) devised an inorganic mixture for use as a colorimetric standard in chlorophyll determinations. This was employed by Ireland (117). Guthrie employed inorganic standards for determinations of chlorophylls *a* and *b*, carotene and carotenol (93).

Scharfnagel (226) studied the component ratio *a/b* in numerous plants by the methods of Willstätter and Stoll (310) and of Guthrie (93).

Seybold and Egle (237) analyzed a large variety of leaf types from many species for chlorophylls *a* and *b* (in ether), carotene (in benzene) and carotenol (in methanol). A visual filter photometer was employed. Although the method is subject to many errors, their analytical results are very interesting, since a wide variation in the ratio of component *a* to *b* and of carotene to carotenol was found for leaves of different species. They also studied (238) the rate of pigment development in etiolated higher plants grown under filtered radiation. Analyses for chlorophylls *a*, *b*, total carotenes, and total carotenols were included.

Sjøberg (247) compared chlorophyll extracts by use of gelatin filters, which are known to be unstable.

Michael (184) and Menke (182) employed a visual filter photometer to estimate total chlorophyll.

Peterson and McKinney (211) employed a modification of Peterson's colorimetric method (210) for a study of tobacco leaves infected with mosaic diseases.

Hilpert and Hofmeier (109) compared absorption spectra of certain copper and potassium derivatives of chlorophyll for colorimetric methods.

Visual spectrophotometry. Visual spectrophotometers were employed by the following: Fleischer (64), with standards $(a+b)$ supplied by Schertz, studied variations in chlorophyll content of *Chlorella* as influenced by nutritive factors; Joslyn and Mackinney

(123) studied the rate of pheophytin formation from chlorophyll due to addition of acids; Hubert (114) followed spectral changes accompanying photo-decomposition of pigment in an acetone extract.

Lubimenko and Hubbenet (164) employed a visual micro-spectroscope for determination of chlorophyll in etiolated seedlings. The band in the red was used, with ethyl chlorophyllide as a standard. They determined chlorophyll and protochlorophyll separately, using the chlorophyll band at 6500–6700 Å and that of protochlorophyll at 6200–6400 Å. This method was developed on a micro scale, only .05 to .1 g. of fresh tissue being required. After treatment with barium hydroxide, which precipitated the chlorophylls, the yellow pigments were determined. Later, Seybold and Egle (239) combined chromatographic separation with colorimetric methods to analyze plant tissues for chlorophylls *a* and *b* separately, carotene and carotenol.

Ghosh and Sen-Gupta (82) attempted to analyze for chlorophylls *a* and *b* in acetone solution. Their standards were prepared by Stoll.

Emerson and Arnold (54) used the 6599 Å neon line with a visual spectrophotometer to measure chlorophyll content of methanol extracts of *Chlorella*. For a standard, a sample of chlorophyll $(a+b)$ prepared from *Chlorella* by Gaffron was employed. This method has since been employed by others (55, 224, 225).

McAlister (178) used Schertz's chlorophyll as a standard to estimate chlorophyll content.

Tottingham and Dutton (295) studied the relative amounts of components *a* and *b* in acetone extracts. The component ratio was found to depend upon the wavelength region to which the leaves had been previously exposed.

Spectography. Heierle *et al.* (101, 299) developed a spectrographic method for the analysis of leaf extracts for chlorophyll *a*, chlorophyll *b*, carotene, and carotenol. Standards for the first two were obtained from Stoll. Karrer supplied carotene, which was mostly *beta*-carotene, and carotenol which was mostly luteol. Absorption curves for the visible region were presented for each of these pigments in ether and for carotene in hexane, carotenol in methyl alcohol, and for chlorophylls in ethyl alcohol. He could detect no deviations from Beer's Law in the concentration range studied for his standard curves. He found that satisfactory analy-

ses could not be made for carotene and carotenol in the presence of the chlorophylls. Therefore, he determined the chlorophylls first on the mixture in ether; he then removed the chlorophylls by saponification, applied the methyl alcohol-hexane fractionation method to separate carotene from carotenol, and determined the latter two separately. Analyses of known quaternary mixtures are given, the percentage error averaging about 5% over a range of -1.33 to $+8.31\%$. Heierle (101) analyzed numerous samples of tobacco leaves by this method. Obviously the carotene and carotenol isomers were not considered and therefore discrepancies could be expected when the method was applied to plant material.

Dastur and Desai (38) studied the chlorophyll content of tropical plants by the spectrographic method of Dastur and Buhariwalla (39). A solid standard $(a+b)$ was prepared and the blue band was used. No account of the yellow pigments appears to have been taken.

Photoelectric filter photometry. Singh and Rao (246), by means of a photoelectric filter instrument, obtained absorption curves for Schertz's chlorophyll $(a+b)$, carotene, and carotenol in 80% methyl alcohol solution. They claim that carotenoids can be detected in chlorophyll preparations in concentrations as low as 0.05%. A serious error is evident in the sensitivity figures given. This is readily apparent by comparison of their figures 1 and 2 (246). Calculations show that this method could not possibly provide such high sensitivity.

Petering, Wolman and Hibbard (209) employed a photoelectric filter instrument to determine total chlorophyll $(a+b)$ in acetone extracts and carotene in petroleum ether solutions. Barium compounds were used to remove chlorophyll from carotene solutions. Five X chlorophyll from American Chlorophyll, Inc. and S. M. A. crystalline carotene were the standards.

Oltman (205) employed a photocell, red filter, and microammeter to estimate chlorophyll concentrations in saponified extracts.

Hellström *et al.* (56, 104) employed a photoelectric method to determine components a and b by measurements made in the red region of absorption. Many experimental details of the method are lacking.

Van Niel and Arnold (297) employed the 6678 Å line of the helium arc for the visual estimation of bacteriopheophytin with an

error not exceeding 3%. Absorption data are given for solutions of bacteriopheophytin in several solvents.

Johnston and Weintraub (122) detected concentrations of chlorophyll as low as 0.01 mg. per l. in acetone extracts with a photometer consisting of a Mazda lamp, red filters, 5 cm. cell, and vacuum thermocouple. Large concentrations of carotenoids did not interfere. A calibration curve was prepared against purified chlorophyll. No account was taken of the *a* to *b* ratio.

Photoelectric spectrophotometry. Zscheile (323) first measured the absorption spectra of chlorophylls *a* and *b* in ether solution by a precise photoelectric method (329). He employed these data in an analytical method (324) for mixtures of components *a* and *b*. He applied this analytical method only to artificial mixtures of his standard preparations. Later, he discussed (326) the possible significance of this method for applications to the study of photosynthesis. Miller, employing an improved apparatus (189), reported (190) quite accurate results (error of 2% or less) for chlorophyll component analysis in unknown quaternary systems when Zscheile's curves were used as standards. This method was employed by Miller and Johnson (192, 193) in analyses of corn leaves from various genetic types.

Johnson and Miller (120) determined total chlorophyll, carotenoids, and *beta*-carotene in two forage grasses. No relationship was found between total chlorophyll or carotenoid and yielding ability. Chlorophyll content was correlated with carotenoid and *beta*-carotene content.

Myers (200) was unable to secure reliable analytical results on algae extracts because the maxima of absorption for his chlorophyll extract were shifted 10–25 Å toward the red with respect to Zscheile's curves. He attributed this shift to the presence of 2.5% ethanol in his ether solutions.

Strain (289) studied development of chlorophyll, carotenes and carotenols in etiolated barley grown under red light.

Mackinney (172) reviewed the more recent spectroscopic values in the literature and pointed out that now four discordant sets of data exist from which one may choose absorption coefficients to employ as standards of purity for spectroscopic analysis. It is hoped that the recent work of Zscheile and Comar (328) will clarify certain preparative and instrumental difficulties which have been

hindrances to previous efforts to reach agreement between different laboratories.

Miscellaneous methods. Deleano and Dick (41) employed a gravimetric method to determine the magnesium of chlorophyll as $MgNH_4AsO_4 \cdot 6H_2O$. This method appears to have a very limited utility.

Fluorescence Spectra and Physiological Applications

The fluorescence spectra of chlorophyll *a* and *b* solutions in ether have been measured by Zscheile (325). The exciting source was the continuous spectrum from a 1000 watt Mazda lamp. The spectrum of *a* contained 2 bands at 6685 and 7230 Å, that of *b* three bands at 6485, 6720, and 7050 Å. The fluorescence maxima for chlorophyll $(a+b)$ in numerous solvents was estimated spectrographically by Baas, Becking and Koning (9). Their method was not very reliable.

Biermacher (16) examined the fluorescence spectra of components *a* and *b* by visual and photographic methods. The maxima of his curves for ether solutions occur at shorter wavelengths than those of Zscheile and others. Biermacher attributed this to autoabsorption of fluorescence within their solutions, which were not as dilute as his solutions. He took special precautions to minimize autoabsorption. His curve for chlorophyll *b* does not contain the middle band found by Zscheile and he ascribed this to contamination of Zscheile's *b* with the *a* component. In the light of the results of Zscheile and Comar (328) it would appear that Zscheile's *b* was certainly not pure. Biermacher also found broad bands at longer wavelengths that 7700 Å, which had previously been overlooked. He studied fluorescence of chlorophyll in many other solvents to obtain band maxima. Some applications were made to leaf fluorescence.

Prins (218) measured the absorption and fluorescence spectra of Stoll's chlorophyll *a* and *b* with a visual spectrophotometer and gave some theoretical attention to fluorescence.

The summarized fluorescence maxima presented by Dhéré (45) indicated to him that in general, the wavelengths of these maxima are related to the refractive index of the solvent. The subject of spectral quality of chlorophyll fluorescence deserves more study.

Stern *et al.* (269, 270, 278 to 281) have reported fluorescence

maxima of many porphyrin derivatives, including the effect of various solvents. Pheophytin *a* exhibits four fluorescence bands in the red and near infrared. A microspectrograph was used, the exciting radiation being blue and ultraviolet mercury arc radiation.

Albers and Knorr (33) reviewed fluorescent properties of chlorophyll solutions. They photographed the fluorescence spectra of solutions of chlorophyll and certain derivatives in four solvents at intervals during prolonged exposure to mercury arc radiation. The pigment components were isolated from Schertz's $a+b$ mixture by the methanol procedure of Willstatter and Stoll (310). These workers extended this study (33) to include the saturation of the solutions with oxygen, carbon dioxide, and nitrogen while the solutions were subjected to photo-decomposition. Fluorescence disappeared as the solutions were bleached. During decomposition the relative intensities of the bands changed but their wavelengths did not shift.

The experiments of Kautsky and coworkers (133 to 138) provided a stimulus for considerable inquiry by Wassink and collaborators (301 to 304) and by Franck and Wood (67) into the physiological factors of leaf fluorescence. Kautsky irradiated fresh leaves in a thermostat with ultraviolet radiation from a mercury arc and measured the fluorescence intensity visually through a red filter. He found an initial burst of fluorescence which rose to a maximum in about one second and then decreased to a low constant value over a 4-minute interval at 30° C. At 0° C. the decrease from the maxima was very slight. The time required to reach the maximum intensity was independent of temperature between 0° and 40° C. However, the time to reach a constant value decreased rapidly with rising temperature.

Later Kautsky *et al.* (131, 139, 140) illuminated fresh leaves with mercury arc radiation of wavelength 3660 Å. A cesium photocell, placed near the leaf, measured the fluorescent radiation (λ 6300–7000 Å) which was filtered through a red filter. The fluorescence intensity was photographically plotted against time. This apparatus permitted more objective measurement in shorter time intervals. Fluorescence was studied as a function of time and temperature. Full fluorescence intensity developed in approximately 3 seconds. The logarithm of fluorescence intensity was directly proportional to time. Damping or quenching of leaf fluorescence by oxygen was studied and theoretical ideas concerning kinetics of photosynthesis

were put forward by Kautsky. Oxygen concentrations of 0.2 to 99% produced considerable damping of fluorescence; that is, the maximum intensity was not maintained but decreased to a constant low value. With low concentrations of .0005 to .04% oxygen, little or no quenching occurred. The interpretation of this work has been criticized by Gaffron (81), Stoll (284), and Emerson (52), partly because the fluorescence was always excited by ultraviolet radiation, a condition which does not represent one of physiological importance, and partly because of the small fraction of absorbed light which is emitted as fluorescence.

In 1938, Kautsky and Eberlein (132) employed visible radiation filtered from a mercury arc and studied damping by oxygen in *Ulva lactuca*. From these experiments they concluded that free oxygen is not the only factor involved in such damping experiments.

Wassink and co-workers (301 to 304) have conducted a very significant series of experiments on the fluorescence of photosynthesizing cells of *Chlorella* and *Chromatium*, in which the work of Kautsky *et al.* has been greatly extended. *Chlorella* exhibits fluorescence because of its chlorophyll content and *Chromatium* because of bacteriochlorophyll. The physical equipment employed for these studies is the best thus far used for such work. For determination of fluorescence spectra, radiation intensities were measured photoelectrically by means of an a. c. amplifier and a cesium photocell, after the radiation passed through a double monochromator. Tests were made to demonstrate that stray or scattered light was not influencing the results. For absorption spectra, a tungsten incandescent lamp was the source. Fluorescence was excited by means of filtered mercury arc radiation of wavelengths 3650, 4358, 5461, and 5790 Å, respectively, as well as with total radiation from this arc and of the region 3000–4000 Å from a carbon arc.

The fluorescence spectrum (301) is essentially the same for living *Chlorella* as for extracted chlorophyll, having a maximum at 6850 Å, a steep decrease on the violet side, an asymmetrical shoulder on the red side, and extending to 7600 Å in the red. In the case of *Chromatium,* the fluorescence spectrum has a maximum in the infrared at 9300 Å, this curve being almost a mirror image of the corresponding curve for *Chlorella, i.e.,* it has a steep decrease to zero on the red side at 9750 Å and a more gradual decrease on the violet side. The fluorescence spectrum of bacteriochlorine extract, however, has two maxima, at 6900 and 8000 Å. In both cases, the

spectral distribution of the fluorescent radiation is independent of the exciting wavelength.

The yield of fluorescence, expressed in ergs per quantum absorbed, was next studied. The cell suspensions were irradiated with monochromatized radiation from the double monochromator, the fluorescence being measured by a photocell, after passing through a filter which absorbed all reflected radiation from the exciting source. Absorption measurements on the suspensions were made by use of an integrating sphere. The technical problems involved in these determinations and associated correction factors are difficult. Results of other workers would be valuable checks on the data, which are very interesting. With *Chlorella* fluorescence, the authors conclude that the yield is independent of wavelength for the region studied, 4500–6400 Å. In the case of *Chromatium* the results are more complicated because of the red pigment system. The fraction of absorbed energy which emerged as fluorescence was 0.15% for *Chlorella* and 0.005% for *Chromatium*.

The relation between CO_2 assimilation and fluorescence in the stationary state (302) was next studied with *Chlorella*. The manometric method was used to measure the gas exchange and filtered continuous radiation excited fluorescence. The fluorescence intensity was directly proportional to the incident intensity, both below and above intensities which were saturating for the gas exchange. Fluorescence was independent of temperature between 12° and 30° C. and of KCN concentrations which inhibited gas exchange. The presence of urethane, however, tended to increase fluorescence slightly. The stationary yield of fluorescence was the same whether pure oxygen or pure nitrogen was used as an atmosphere, due to O_2 production during assimilation. It was concluded that agents which influence only the Blackman-reaction do not affect fluorescence. When fluorescence is affected, the photochemical part of the process must be altered. A mathematical treatment and interpretation of these results was presented (303).

Wassink and Katz (304) next undertook a study of the simultaneous measurements of fluorescence and gas exchange in *Chlorella* during the first few minutes of light exposure. Rapid galvanometer readings were taken to study the course of the fluorescence. The general type of fluorescence curve showed considerable detail, as indicated in Figure 3 taken from (304).

The effects of KCN, O_2, NaHS, and age of culture were then studied in relation to the various parts of this curve. These authors, in agreement with Kautsky, also found an influence of oxygen upon fluorescence. However, they considered this effect of only secondary importance for the energy transfer from chlorophyll in photosynthesis. The original papers must be consulted for details of these theories. Emerson (52), Stoll (284) and Gaffron (79, 80) have discussed in detail the relation of O_2 to the kinetics of photosynthesis.

Gaffron (81) has abandoned hope of tracing the course of light reactions in photosynthesis by measurement of fluorescence because of the very small fraction of all activated chlorophyll molecules which actually show fluorescence.

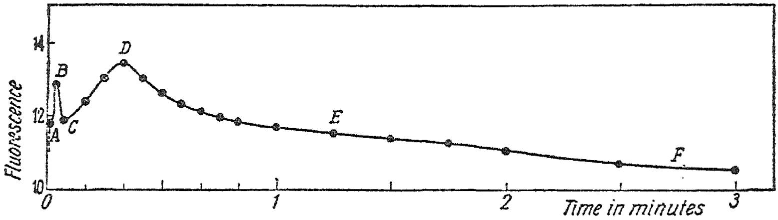


FIG. 3. General type of fluorescence-time-relation in *Chlorella*. The light is admitted at time zero [from Wassink and Katz (304)].

Knorr and Albers (144) photographed the fluorescence spectra of chlorophyll *a* solutions, in ether, benzene, acetone, and methanol, as produced by mercury arc excitation. In ether, maxima were found at 6718, 6330, 7335, and 6792 Å. Solutions of chlorophyll *b* (145) were studied also. Changes due to decomposition were followed. Fluorescence and absorption were followed during reduction and reoxidation of chlorophyll solutions (5). Differences were found when carbon dioxide was used instead of nitrogen as an atmosphere. The effect of solvent and atmosphere upon the photodecomposition of chlorophyll *a*, as shown by fluorescence changes, was studied (146) for benzene and acetone under oxygen, carbon dioxide, and nitrogen.

Franck and Teller (66) discussed fluorescence and light absorption in relation to units of chlorophyll.

McAlister (178) used a very rapid spectrographic method to study the CO_2 relations during photosynthesis in wheat. He discussed his very interesting findings in relation to fluorescence of chlorophyll in leaves and to the "chlorophyll unit."

Optical Activity

Stoll and Wiedemann (285) determined the optical activity of solutions of chlorophylls *a* and *b* and certain derivatives. They employed a carbon arc, monochromator, and polarimeter. The matched fields were photographed. Wavelength 7200 Å was used to obtain sufficient transmission through concentrated solutions. Specific rotations of $-262°$ and $-267°$ were obtained from components *a* and *b* respectively at 25° C. Upon standing, solutions lost this optical activity. It will be seen in the formula for chlorophyll *a* above that there are three asymmetric carbon atoms which could be responsible for the observed activity.

Film Properties

Hansen (96) applied the film balance technique to the problem of measurement of areas of chlorophyll monomolecular films. He reported a surface area of 106 sq. Å for the chlorophyll nucleus of 4 pyrrole groups around the Mg atom. This is in good agreement with the assumption that these groups are situated in one plane, for calculations based on interatomic distances give a value of 100–110 sq. Å. X-ray data, although very incomplete, can be explained by the same arrangement of groups. It was reported (97) that X-ray measurements on ethyl chlorophyllide indicate that the surface of the porphin nucleus is much larger than the apparent surface shown by films. Ketelaar and Hanson (141) studied the elementary cell and space group of ethyl chlorophyllide crystals.

Nicolai and Weurman (201) have studied properties of multifilms composed of successive layers of chlorophyll, and of chlorophyll alternating with globin and lecithin compounds, "planticin" and "ovolecithin." The absorption properties of these films for filtered red light were measured with a selenium cell, some differences being found for different types of films. None of these film preparations showed fluorescent properties as did the chlorophyll-globin adsorbates prepared by Noack (202).

Bakker (11) studied the stability and fluorescence of chlorophyll and pheophytin suspensoids. He found these to be negatively charged, hydrophobic suspensoids. An optical method was used to measure degrees of flocculation. A fluorescent solution and coacervate of lecithin with chlorophyll was obtained.

Protochlorophyll

The precursor of chlorophylls *a* and *b* is the subject of considerable debate in the literature and little highly accurate work has been done on the subject, since the experimental difficulties are very great. Noack and Kiessling (203, 204) regard protochlorophyll as the precursor of chlorophyll *a*, which is formed from protochlorophyll by the action of light. They presented visual observations of the wavelengths and relative intensities of the absorption bands of protochlorophyll and protopheophytin in chloroform-pyridine solutions. Lubimenko and Gortikowa (163) stated that there are two spectroscopically different forms of protochlorophyll, corresponding to chlorophylls *a* and *b*. Rudolph (222) determined molar extinction coefficients for chlorophylls *a* and *b*, carotene, and carotenol and relative extinction coefficients for protochlorophyll with a visual spectrophotometer. Analyses were made for these compounds in a study of pigment development in etiolated leaves. Michael (184) and Scharfnagel (226) studied chlorophyll development in plants.

Later, Kar (124) employed the same method for the study of pigments purified by adsorption technique. He compared the molecular absorption coefficients with those of other workers for ethyl chlorophyllide prepared by H. Fisher and for *beta*-carotene and luteol prepared by Zechmeister. His values were higher than previously reported values. These were used in an extensive study of pigment content as related to photoperiodism.

Seybold (236) employed the adsorption method with powdered sucrose to separate the components of a petroleum ether extract of protochlorophyll into a blue-green and a yellow-green component (protochlorophylls *a* and *b*, respectively). Sketches of the absorption spectra of ether solutions were presented as observed visually. The fluorescence of *a* was stated to be stronger than that of *b*, in the region 6200 to 6550 Å, in agreement with Noack and Kiessling. Seybold characterized protocarotene and protocarotenol also. Later, Seybold and Egle (240) revised the spectra of protochlorophylls *a* and *b*, having purified their samples more rigorously. They also studied the protopheophytin and protochlorophyllide derivatives of these compounds. All of these observations lack the precision of quantitative treatment that would make the protochlorophyll system more convincing.

Dhéré (44) found a single fluorescence maximum of protochlorophyll at 6430 Å in methyl alcohol and at 6265 Å in ether, using the spectrographic method.

Rothemund (33) reviewed the protochlorophyll problem and presented spectroscopic observations on certain plant extracts and chlorophyll derivatives.

Myers (200) reported the formation of chlorophyll *b* (or a very similar substance) as well as chlorophyll *a*, in the dark by certain algae.

Bacterial Photosynthetic Pigments

Since certain bacteria are able to carry on photosynthesis, the pigments found in them are of equal theoretical interest to chlorophyll as found in higher plants. The structural chemistry of bacteriochlorophyll is discussed in Fischer and Lambrecht's work (62). Of particular interest is the fact that these organisms are able to utilize infrared radiation for photosynthesis. The spectroscopic properties of bacterial pigments have been studied considerably during the past five years.

Ehrisman and Noethling (49) employed a double monochromator and photoelectric method to study the absorption spectra of pyocyanin, prodigiosin and violacein. van Niel and Smith (298) determined absorption maxima for solutions of spirilloxanthin in numerous solvents and measured the spectrum from 4000 to 6000 Å in carbon disulfide by the photoelectric method. Reliable results in the ultraviolet were not obtained because of the extreme sensitivity of this pigment to ultraviolet radiation. This is probably a result of the high degree of unsaturation of the spirilloxanthin molecule, which has 15 double bonds.

Wassink *et al.* (301) extracted the pigments of *Chromatium*. They found that bacteriochlorine absorbs most near 5000 Å and bacteriopurpurine near 8000 Å. These pigments are also very photolabile and reproducible spectra can be obtained only in the presence of an active reducing agent, such as H_2S, and in the absence of O_2 and excessive light. The bacteria themselves absorb between 8000 and 9000 Å.

Wassink *et al.* (305) theorized on the resolution of absorption band systems of various bacterial strains into separate symmetrical bands. They concluded that the infrared absorption spectra of purple bacteria suspensions vary between strains because the bac-

teriochlorophyll, common to all, is bound to different proteins within the cell.

Seybold and Egle (241) isolated bacteriochlorophylls *a* and *b* by the adsorption technique. These components have colors in solution similar to the corresponding chlorophylls. However, ether solutions of bacteriochlorophyll *a* do not fluoresce. The absorption spectra presented are not quantitative, since only the visual method was used. The spectra of the corresponding pheophytins in ether solution were also indicated from visual estimation.

In a study on the quantum yield of hydrogen and CO_2 assimilation in *Streptococcus varians,* French (72) photographed the infrared absorption bands of this organism which occur at approximately 8000 and 8600 Å. In the case of *Spirillum rubrum* (73) these corresponding bands are at 8000 and 8800 Å. These findings may indicate different pigments in different species. French found that upon extraction with methyl alcohol, the 8800 Å band was shifted to 7700 Å. The yellow band shifted in the opposite direction from 5900 to 6050 Å. A photoelectric spectrophotometer was used for comparison of spectra of live and bleached bacteria. French concluded that only the green bacteriochlorophyll and not the red pigment, can absorb radiation for photosynthesis.

To obtain a solution of the pigment-protein complex from photosynthetic bacteria, French (74) used high frequency vibrations in the supersonic range. The absorption spectrum of such extracts was studied. Maximum absorption occurred near 5000 and 8800 Å. French concluded that this extract is closely similar in light absorbing properties to the intact pigment within the cell. Both the bacteriochlorophyll and spirilloxanthin are attached to the same or similar proteins, since they could not be resolved by the Tswett adsorption method (75). These supersonic extracts will act as photocatalysts for the oxidation of ascorbic acid with either visible or infrared radiation (76). French then (77) examined the absorption spectra in the region 4500 to 9500 (or 10,000) Å of the supersonic extracts of four species of purple bacteria. All showed maxima at 5900 and 7900 Å and also at another wavelength which varied with the species (8400, 8550, or 8750 Å). Bacteriopheophytin has absorption maxima at 5300 and 7500 Å. French concluded that the extraction of cell juice by supersonic vibrations does not change the position of absorption bands or radiation absorbing capacity of the pigment.

FUNCTION IN PLANTS

In 1930, in a review on the rapidly developing chemistry of carotenoid pigments, Smith (251) wrote that "almost every conceivable physiological function has been ascribed to the yellow pigments and yet not one clearly demonstrated." Although much more has been learned during the last decade concerning the chemical and physical properties and occurrence of carotenoids, we remain ignorant of their function in the life of the plant.

Mention should be made of the comparative studies of Carter, Heilbron, and Lythgoe (27) on the pigment content of algae in relation to their classification and resemblances to higher plants. Tilden (294) classified the algae with special reference to pigmentation.

Smith and Morgan (254) concluded that chlorophyll need not be present in fruits previous to the development of carotene or lycopene.

Bünning (24) studied the phototropic response curve of *Phycomyces* for various wavelengths of filtered light. Attempts were made to correlate this response with the absorption of carotenoids and chlorophyll.

It is extremely interesting to note that the phototropic sensitivity curve of oat coleoptile for various wavelengths, as determined by Johnston (121), follows very closely in general shape and position of maxima and minimum the absorption curve for *alpha*-carotene. No satisfactory explanation for this coincidence has been proposed.

It is to be expected that trustworthy analyses on many of these pigments, correlated with physiological conditions of the plant, will make possible more plausible theories as to the function of these pigments in plant metabolism. In recent years more emphasis is being placed upon the spectroscopic and photochemical aspects of plant pigments in relation to their substrates and this trend will doubtless continue.

The question of the possible rôle of carotenoids in photosynthesis has been discussed by Seybold (235), Montfort (195) and Emerson (53).

The Annual Reports of the Division of Plant Biology, Carnegie Institution of Washington (256) summarized much information on physical properties of pigments and their relation to biological problems.

PREVIOUS REVIEWS—VIEWPOINTS

Since the subject matter of this review is very restricted, it seems desirable to refer the reader to other articles of related or more general interest and which are (at least partially) of the review type.

Late in 1938, Weir (307) reviewed the subject of "The Structure of the Chloroplast" in this journal. Since Weir devoted a section to the submicroscopic structure of the chloroplast, the writer has not considered in the present review many papers dealing with the state of chlorophyll within the leaf. Three optical effects, fluorescence, light absorption, and double refraction have been extensively applied to chlorophyll-containing bodies within the cell to obtain evidence on this subject.

Strain's monograph, "Leaf Xanthophylls" (290), contains much helpful comparative and preparative information on carotenoid pigments, particularly in regard to their chemical and physical properties. Zechmeister's monograph, "Carotinoide" (316), outlines in detail the properties of carotenoids as of 1934. It is illustrated with photomicrographs of carotenoid crystals. See also Zechmeister's contribution to Klein's "Handbuch der Pflanzenanalyse" (143). The volume by Zechmeister and Cholnoky (317) includes chlorophylls and carotenoids in a detailed discussion of the application of the adsorption method to many classes of organic compounds. Bogert's chapter on Carotenoids (19) in Gilman's Organic Chemistry is especially good from the chemical viewpoint.

Stoll and Wiedemann (286) presented a very extensive review of progress made in the chemistry of chlorophyll from 1904 to 1938. A large bibliography is included. Bacteriochlorophyll, protochlorophyll, and the state of chlorophyll in the leaf are discussed.

Dhéré (45) has recently reviewed the spectrochemistry of fluorescence of biological products and included chlorophylls, carotenoids, and related compounds. He has summarized the work of many authors and presents an excellent bibliography.

In a paper entitled "Criteria for Purity of Chlorophyll Preparations" (172), Mackinney discussed the subject of chlorophyll preparation, especially from the chromatographic and spectroscopic viewpoints. His simplified procedure was reported and spectroscopic values were given.

Emerson (53), Gaffron (81), and Mackinney (173) have con-

tinued the excellent reviews on subjects relating to plant pigments presented frequently in the Annual Review of Biochemistry. The first two deal with photosynthesis, the last with "Plant Pigments," including both chlorophylls and carotenoids with emphasis on their chemistry.

Deleano and Dick presented a detailed review (40) of methods for carotenoid identification and determination. This review is very good for the older references of more historical interest.

The two volumes on "Biological Effects of Radiation" (47), edited by Duggar, contain much information on plant pigments, especially as related to radiation. Many specialized subjects are discussed here.

Manning's review on "Photosynthesis" (175) emphasizes the photochemical and kinetic aspects of photosynthesis. Relations with physical properties of pigments are discussed.

The third Cold Spring Harbor Symposium on Quantitative Biology (33) contains numerous papers on various phases of photosynthesis, especially relative to chlorophyll. A paper by K. Meyer (33) summarizes work by Meyer, Noack and Gaffron on photosensitized oxidation of ethylenic double bonds.

Miller (191) summarized much of the spectroscopic work reported up to 1939 on plant pigments studied by the spectrophotoelectric method. He has referred in detail to many experimental points involved in this method of measurement.

Steele (263) reviewed "Recent Progress in Determining Chemical Structure of Chlorophyll" up to 1937. See also the section by Treibs (143) on Chlorophyll. Hubert (116) reviewed the optical approach to the problem of the state of chlorophyll in the living leaf as of 1935. He presented original data on colloidal suspensions epared to simulate leaf conditions.

Literature Cited

Adam Hilger, Ltd. London, England. Nutting colorimeter.

———. Spekker photoelectric absorptiometer.

Albers V. M., and Knorr, H V. The absorption spectra of solutions of pure chlorophyll and of chloroplasts in living cells. Science **85**: 51–52. 1937.

———, and ———. Absorption spectra of single chloroplasts in living cells in the region from 664 mμ to 704 mμ. Pl. Physiol. **12**: 833–843. 1937.

———, ———, and Rothemund, P. Fluorescence of the chlorophyll series: The reversible reduction of chlorophyll *a* and *b*. Physiol. Rev. **47**: 198. 1935.

American Instrument Co. Silver Spring, Maryland. Neutral wedge photometer.

7. ————. Type V photoelectric photometer.
8. Arthur H. Thomas Co. Philadelphia, Pa. Klett-Summerson photoelectric colorimeter.
9. Baas Becking, L. G. M., and Koning, H. C. Preliminary studies on the chlorophyll spectrum. Proc. Kon. Ak. Amst. **37**: 674–678. 1934.
10. Bakker, H. A. Purification of chlorophyll. Proc. Kon. Ak. Amst. **37**: 679–684. 1934.
11. ————. Notes on the colloid chemistry of chlorophyll. Proc. Kon. Ak. Amst. **37**: 688–694. 1934.
12. Barnett, H. M. The determination of carotene in butter fat. Jour. Biol. Chem. **105**: 259–267. 1934.
13. Batty, J. W., Heilbron, I. M., and Jones, W. E. Studies in the polyene series. Jour. Chem. Soc. **329**: 1556–1560. 1939.
14. Baxter, J. G., and Robeson, C. D. Crystalline vitamin A palmitate and vitamin A alcohol. Science **92**: 203–204. 1940.
15. Benne, E. J., Wolman, W., Hibbard, R. P., and Miller, E. J. A comparison of the Petering-Wolman-Hibbard procedure for determining carotene, and two modifications thereof with the Peterson-Hughes-Freeman technic. Jour. Assoc. Official Agr. Chem. **23**: 709–716. 1940.
16. Biermacher, O. The visible and infra-red fluorescence spectra of chlorophyll *a*, chlorophyll *b*, and several porphyrins. Thesis, Univ. Freibourg, Switzerland. 1936.
17. Bills, C. E., and McDonald, F. G. The carotene content of ten varieties of carrots. Science **76**: 108. 1932.
18. Blaydes, G. W. Preserving the natural color of green plants. Science **85**: 126–127. 1937.
19. Bogert, M. T. Carotenoids: The polyene pigments of plants and animals. Organic Chemistry, by Gilman, H.: pp. 1138–1219. 1938.
20. Bowden, F. P., and Morris, S. D. D. Physico-chemical studies of complex organic molecules. Part II. Absorption spectra at low temperatures. Proc. Roy. Soc. B **115**: 274–278. 1934.
21. Brice, A. A compensating circuit for blocking layer photoelectric cells. R. S. I. **8**: 279–285. 1937.
22. Brooke, R. O., Tyler, S. W., and Baker, W. S. Determination of beta-carotene in alfalfa meals. Ind. Eng. Chem., Anal. Ed. **11**: 104–106. 1939.
23. Bruins, H. R., Overhoff, J., and Wolff, L. K. The molecular weight of vitamin A. Biochem. Jour. **25**: 430–438. 1931.
24. Bünning, E. Phototropismus und Carotinoide. III. Weitere Untersuchungen an Pilzen und höheren Pflanzen. Planta **27**: 583–610. 1938.
25. Buxton, L. O. Carotenoids in yellow corn. Ind. Eng. Chem., Anal. Ed. **11**: 128–129. 1939.
26. Buxton, L. O., and Dombrow, B. A. The carotenoids in forage. Ind. Eng. Chem., Anal. Ed. **10**: 262–263. 1938.
26*a*. Carter, G. P., and Gillam, A. E. The isomerization of carotenes. III. Reconsideration of the change β-carotene to ψα-carotene. Biochem. Jour. **33**: 1325–1331. 1939.
27. Carter, P. W., Heilbron, I. M., and Lythgoe, B. The lipochromes and sterols of the algal classes. Proc. Roy. Soc. London B **128**: 82–108. 1939.
28. Central Scientific Co. Chicago, Ill. Cenco photoelectric spectrophotometer.
29. ————. Cenco-Sheard-Sanford photolometer.
30. Chicago Apparatus Co. Chicago, Ill. Schoenberg electric colorimeter.
31. Clark, G. L., and Gring, J. L. Carotenoids in yellow corn. Ind. Eng. Chem., Anal. Ed. **9**: 271–274. 1937.
32. Clausen, S. W., and McCoord, A. B. The determination of carotene

and xanthophyll by a single distribution between liquid phases. Jour. Biol. Chem. 113: 89–104. 1936.
33. Cold Spring Harbor Symposia on Quantitative Biology III. Long Island Biological Association. 1935.
34. Coleman Electric Co., Inc. Maywood, Ill. The Coleman regional spectrophotometer.
35. Conant, J. B., and Kamerling, S. E. Studies in the chlorophyll series. VII. Evidence as to structure from measurements of absorption spectra. Jour. Am. Chem. Soc. 53: 3522–3529. 1931.
36. Connor, C. L. Studies on lipochromes. II. The identification of carotin, xanthophyll, and associated lipoids in tissues. Amer. Jour. Path. 4: 235–244. 1928.
37. Dániel, E., and Scheff, G. J. An improved method for determination of blood carotene. Proc. Soc. Expt. Biol. & Med. 33: 26–30. 1935.
38. Dastur, R. H., and Desai, B. L. The relation between water-content, chlorophyll-content, and the rate of photosynthesis in some tropical plants at different temperatures. Ann. Bot. 57: 69–88. 1933.
39. ———, and Buhariwalla, N. A. Chlorophyll from tropical plants and its quantitative determination by means of the spectrograph. Ann. Bot. 42: 949–964. 1928.
40. Deleano, N. T., and Dick, J. Beiträge zur Kenntnis des Carotins. Neue Methoden zur Darstellung, Nachweis und Bestimmung. Bul. Soc. Chimie România 14: 117–170. 1932.
41. Deleano, N. T., and Dick, J. Eine neue Methode zur Bestimmung des Chlorophylls. Biochem. Zeits. 268: 317–321. 1934.
42. Deuber, C. G. Mineral nutrition and chlorophyll development in seedlings. Am. Jour. Bot. 15: 271–276. 1928.
43. ———. Chlorophyll in tree leaves. Jour. For. 27: 76–77. 1929.
44. Dhéré, Ch. Sur le spectre de fluorescence de la protochlorophylle. Comp. Rend. Acad. Sci. 192: 1496–1499. 1931.
45. ———. La spectrochimie de fluorescence dans l'étude des produits biologiques. Forts. Chemie Org. Nat. II. 301–341. 1939.
46. ———, and Biermacher, O. Sur la purification et le spectre de fluorescence de la chlorophylle *b*. Comp. Rend. Seances Soc. Biol. 122: 591–594. 1936.
47. Duggar, B. M. Biological effects of radiation. 1936.
48. Egle, K. Über die Gültigkeit der Kundt'schen Regel bei Chlorophyllspektren. Sitzungsb. Heidelb. Akad. Wiss. 1: 19–29. 1939.
49. Ehrisman, O., and Noethling, W. Die Absorptionsspektren des Pyocyanins, Prodigiosins und Violaceins. Biochem. Zeits. 284: 376–382. 1936.
50. E. Leitz, Inc. New York City. Leifo photometer.
51. Embree, N. D., and Shantz, E. M. Cyclization of vitamin A_2. Jour. Biol. Chem. 132: 619–626. 1940.
52. Emerson, R. Recent investigations of chlorophyll photosynthesis. Ergebn. Enzym. 5: 305–347. 1936.
53. ———. Photosynthesis. Ann. Rev. Biochem. 6: 535–553. 1937.
54. ———, and Arnold, W. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. 16: 191–204. 1932.
55. ———, Green, L., and Webb, J. L. Relation between quantity of chlorophyll and capacity for photosynthesis. Pl. Physiol. 15: 311–317. 1940.
56. Euler, H. v., Bergman, B., and Hellström, H. Über das Verhältnis von Chloroplastenzahl und Chlorophyllkonzentration bei *Elodea densa*. Ber. Deut. Bot. Ges. 52: 458–462. 1934.
57. Ferguson, W. S., and Bishop, G. The estimation of carotene in agricultural products. The Analyst 61: 515–518. 1936.
58. Ferrari, C. G. Spectrophotometric determination of the carotinoid pigment content of wheat flour. Cereal Chemistry 10: 277–286. 1933.

59. ———, AND BAILEY, C. H. Carotinoid pigments of flour. Cereal Chemistry 6: 218–240. 1929.
60. ———, AND ———. The determination of carotin in flour. Cereal Chemistry 6: 347–371. 1929.
61. FISCHER, H. Zur Kenntnis von Chlorophyll. Annalen 537: 170–177. 1939.
62. ———, AND LAMBRECHT, R. Über Bacteriochlorophyll *a*. Zeits. Physiol. Chem. 249: I–III. 1937.
63. FISHER SCIENTIFIC Co. Pittsburgh, Penn. Fisher electrophotometer.
64. FLEISCHER, W. E. The relation between chlorophyll content and rate of photosynthesis. Jour. Gen. Physiol. 18: 573–597. 1935.
65. FOX, D. L. Carotenoids and other lipoid-soluble pigments in the sea and in deep marine mud. Proc. Nat. Acad. Sci. 23: 295–301. 1937.
66. FRANCK, J., AND TELLER, E. Migration and photochemical action of excitation energy in crystals. Jour. Chem. Physics 6: 861–872. 1938.
67. ———, AND WOOD, R. W. Fluorescence of chlorophyll in its relation to photochemical processes in plants and organic solutions. Jour. Chem. Phys. 4: 551–560. 1936.
68. FRAPS, G. S., KEMMERER, A. R., AND GREENBERG, S. M. Factors affecting adsorptive power of magnesia for carotene. Ind. Eng. Chem., Anal. Ed. 12: 16–18. 1940.
69. ———, ———, AND ———. Determination of carotene in presence of lycopene. Jour. Soc. Official Agr. Chem. 23: 422–425. 1940.
70. ———, ———, AND ———. An adsorption method for the determination of pure carotene. Jour. Assoc. Official Agr. Chem. 23: 659–662. 1940.
71. ———, TREICHLER, R., AND KEMMERER, A. R. Relation of the carotene content of certain feed materials to their vitamin A potency. Jour. Agr. Res. 53: 713–716. 1936.
72. FRENCH, C. S. The quantum yield of hydrogen and carbon dioxide assimilation in purple bacteria. Jour. Gen. Physiol. 20: 711–735. 1937.
73. ———. The rate of CO_2 assimilation by purple bacteria at various wave lengths of light. Jour. Gen. Physiol. 21: 71–87. 1937.
74. ———. The chromoproteins of photosynthetic purple bacteria. Science 88: 60–62. 1938.
75. ———. The chromoproteins of photosynthetic bacteria. Jour. Biol. Chem. 123: 38. 1938.
76. ———. The pigment-protein compound in photosynthetic bacteria. I. The extraction and properties of photosynthin. Jour. Gen. Physiol. 23: 469–481. 1940.
77. ———. The pigment-protein compound in photosynthetic bacteria. II. The absorption curves of photosynthin from several species of bacteria. Jour. Gen. Physiol. 23: 483–494. 1940.
78. FROBER-FAYBOR Co. Cleveland, Ohio. Frober-Faybor photo-electric colorimeter.
79. GAFFRON, H. Inwiefern ist Sauerstoff für die Kohlensäureassimilation der grünen Pflanzen unentbehrlich? Naturwiss. 23: 528. 1935.
80. ———. Über die Unabhängigkeit der Kohlensäureassimilation der grünen Pflanzen von der Anwesenheit kleiner Sauerstoffmengen und über eine reversible Hemmung der Assimilation durch Kohlenoxyd. Biochem. Zeits. 280: 337–359. 1935.
81. ———. Chemical aspects of photosynthesis. Ann. Rev. Biochem. 8: 483–501. 1939.
82. GHOSH, J. C., AND SEN-GUPTA, S. B. Studies in the optical properties of chlorophyll. I. Absorption of light by solutions and suspensions of chlorophyll-α and chlorophyll-β and their mixtures. Jour. Indian. Chem. Soc. 8: 581–589. 1931.

83. GILLAM, A. E. Spectrometric measurements on various carotenoids, Biochem. Jour. **29**: 1831–1836. 1935.
84. ———, HEILBRON, I. M., JONES, W. E., LEDERER, E., BATTY, J. W. AND BEYNON, J. H., AND ROSANOVA, V. On the occurrence and constitution of the 693 mμ chromogen (vitamins A_2?) in fish liver oils, Biochem. Jour. **32**: 405–416. 1938.
85. ———, ———, MORTON, R. A., AND DRUMMOND, J. C. The isomerization of carotene by means of antimony trichloride. Biochem. Jour. **26**: 1174–1177. 1932.
86. ———, AND RIDI, M. S. E. Adsorption of grass and butter carotenes on alumina. Nature **136**: 914–915. 1935.
87. ———, AND ———. The isomerization of carotenes by chromatographic adsorption. I. Pseudo-α-carotene. Biochem. Jour. **30**: 1735–1742. 1936.
88. ———, AND ———. The variation of the extinction coefficient of vitamin A with solvent. Biochem. Jour. **32**: 820–825. 1938.
89. ———, ———, AND KON, S. K. The isomerization of carotenes by chromatographic adsorption. II. Neo-α-carotene. Biochem. Jour. **31**: 1605–1610. 1937.
90. GOLDBLATT, H., AND BARNETT, H. M. Carotene and vitamin A. Proc. Soc. Exp. Biol. & Med. **30**: 201–204. 1932.
91. GUILBERT, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. Ind. Eng. Chem., Anal. Ed. **6**: 452–453. 1934.
92. GUTHRIE, J. D. A stable colorimetric standard for chlorophyll determinations. Amer. Jour. Bot. **15**: 86–87. 1928.
93. ———. Effect of environmental conditions on the chloroplast pigments. Amer. Jour. Bot. **16**: 716–746. 1929.
94. HAGENBACH, A., AUERBACHER, F., AND WIEDEMANN, E. Zur Kenntnis der Lichtabsorption von Porphinfarbstoffen und über einige mögliche Beziehungen derselben zu ihrer Konstitution. Helvetica Phys. Acta **9**: 3–26. 1936.
95. HAND, D. B., AND SHARP, P. F. Rapid photoelectric determination of carotenoid in milk fat. Jour. Dairy Sci. **22**: 729–735. 1939.
96. HANSON, E. A. Notes on some physical properties of chlorophyll films. Proc. Kon. Ak. Amst. **40**: 281–284. 1937.
97. ———, MEEUSE, A. D. J., MOMMAERTS, W. F. H. M., AND BASS BECKING, L. G. M. A note on the chlorophyll-contents of the granum. Chron. Bot. **4**: 104–105. 1938.
98. HARRIMAN, P. A. Effect of various methods of storage on chlorophyll content of leaves. Pl. Physiol. **5**: 599–606. 1930.
99. HAUSSER, K. W., KUHN, R., AND SEITZ, G. Lichtabsorption und Doppel-bindung V. Über die Absorption von Verbindungen mit konjugierten Kohlenstoffdoppelbindungen bei tiefer Temperatur. Zeits. Physik. Chemie (B) **29**: 391–416. 1935.
100. ———, ———, SMAKULA, A., AND KREUCHEN, K. H. Lichtabsorption und Doppelbindung I. Aufgaben und Methoden. Zeits. Physik. Chemie (B) **29**: 363–370. 1935.
101. HEIERLE, E. Blattfarbstoffuntersuchungen an einer grün- und gelbblättrigen Tabakvarietät. Ber. Schweiz. Bot. Ges. **44**: 17–86. 1935.
102. HEILBRON, I. M., JOHNSON, A. W., AND JONES, W. E. Studies in the polyene series. Part IV. Jour. Chem. Soc. **330**: 1560–1563. 1939.
103. ———, JONES, W. E., AND SPINKS, A. Studies in the polyene series. Part II. Jour. Chem. Soc. **328**: 1554–1556. 1939.
104. HELLSTRÖM, H., AND BURSTRÖM, D. Über das Komponentenverhältnis des Chlorophylls in chlorophylldefekten Mutanten. Biochem. Zeits. **258**: 221–227. 1933.
105. HENRICI, M. Eleventh report of director of veterinary education and research. Part I. Dept. Agr., Union So. Afr. p. 259. 1927.

106. Hickman, K. C. D. Molecular distillation. State of the vitamins in certain fish liver oils. Ind. Eng. Chem. **29**: 1107–1111. 1937.
107. Hicks, P. A., and Panisset, T. E. The quantitative determination of minute amounts of chlorophyll. New Phytol. **33**: 199–210. 1934.
108. Hilbert, G. E., and Jansen, E. F. A study of the absorption spectra of some carotenoid pigments at liquid air temperatures and its applications to the carotenoid pigments of cow-pea leaves (*Vigna sinensis*). Jour. Biol. Chem. **106**: 97–105. 1934.
109. Hilpert, S., and Hofmeier, H. Über dem örtlichen Nachweis und die quantitative Bestimmung von Chlorophyll in Pflanzenteilen. Ber. **66B**: 1443–1445. 1933.
110. Hodgson, R. E. The relation of color and carotene content of roughage in the dairy ration to the color, carotene content, and vitamin A activity of butterfat. Jour. Agr. Res. **57**: 513–528. 1938.
111. Holmes, H. N., and Bromund, W. H. Bixin solutions as colorimetric standards for the determination of carotene. Jour. Biol. Chem. **112**: 437–441. 1936.
112. ———, and Corbet, R. E. The isolation of crystalline vitamin A. Jour. Am. Chem. Soc. **59**: 2042–2047. 1937.
113. ———, Lava, V. G., Delfs, E., and Cassidy, H. G. Comparative studies on the adsorption behavior of crude vitamin A, carotene, and cholesterol. Jour. Biol. Chem. **99**: 417–427. 1933.
114. Hubert, B. On the photodecomposition of chlorophyll. Proc. Kon. Ak. Amst. **37**: 684–687. 1934.
115. ———. Estimation of the band position of chlorophyll in different media. Proc. Kon. Ak. Amst. **37**: 695–700. 1934.
116. ———. The physical state of chlorophyll in the living plastid. Rec. Trav. Bot. Néerl. **32**: 323–390. 1935.
117. Ireland, J. C. Heritable variations in chlorophyll. Pl. Physiol. **13**: 863–865. 1938.
118. Johnson, I. J., and Miller, Elmer S. Variation in carotenoid pigment concentration among inbred and crossbred strains of corn. Cereal Chemistry **15**: 345–350. 1938.
119. ———, and ———. Immediate effect of cross pollination on the carotenoid pigments in the endosperm of maize. Cereal Chemistry **16**: 88–92. 1939.
120. ———, and ———. Leaf pigment concentration and its relation to yield in Fairway Crested wheat grass and Parkland Brome grass. Jour. Amer. Soc. Agron. **32**: 302–307. 1940.
121. Johnston, E. S. Phototropic sensitivity in relation to wave length. Smithsonian Misc. Coll. **92**(11): 1–17. 1934.
122. ———, and Weintraub, R. L. The determination of small amounts of chlorophyll—Apparatus and method. Smithsonian Misc. Coll. **98**(19): 1–5. 1939.
123. Joslyn, M. A., and Mackinney, G. The rate of conversion of chlorophyll to pheophytin. Jour. Am. Chem. Soc. **60**: 1132–1136. 1938.
124. Kar, B. K. Über das Verhalten der Plastidfarbstoffe photoperiodisch reagierender Pflanzen bei verschiedenem Lichtgenuss. Planta **26**: 420–462. 1937.
125. Karrer, P. Chemistry of vitamins A and C. Chem. Rev. **14**: 17–30. 1934.
126. ———, and Solmssen, U. Die Carotinoide der Purpurbakterien. I. Helvet. Chim. Acta **18**: 1306–1315. 1935.
127. ———, and ———. Die Carotinoide der Purpurbakterien. II. Über Rhodoviolascin. Helv. Chim. Acta **19**: 3–5. 1936.
128. ———, and ———. Carotinoide aus Purpurbakterien. III. Helv. Chim. Acta **19**: 1019–1024. 1936.
129. ———, and Straus, W. Eigenschaftenkolloider Carotinlösungen. Helv. Chim. Acta **21**: 1624–1636. 1938.

130. KATZ, E., AND WASSINK, E. C. Infrared absorption spectra of chlorophyllous pigments in living cells and in extra-cellular states. Enzymol. 7: 97–112. 1939.
131. KAUTSKY, H. Die Wechselwirkung zwischen Sensibilisatoren und Sauerstoff im Licht. Biochem. Zeits. **291**: 271–284. 1937.
132. ———, AND EBERLEIN, R. Neue Messungen der Fluorescenz—Intensitäts—Änderungen grüner Pflanzen. Naturwiss. **26**: 576–577. 1938.
133. ———, ET AL. Energie-Umwandlungen an Grenzflächen. VI. Mitteil: Kohlensäure-Assimilation. Ber. **65**: 1762–1770. 1932.
134. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. I. Das Fluoreszenzverhalten grüner Pflanzen. Biochem. Zeits. **274**: 423–434. 1934.
135. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. II. Mitteilung: 1. Apparatur zur vergleichenden Messung der Fluoreszenzänderungen lebender Blätter. 2. Der Einfluss der Temperatur auf die Fluoreszenzkurve. Biochem. Zeits. **274**: 435–451. 1934.
136. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. III. Mitteilung: Der Einfluss der Kohlensäure auf das Fluoreszenz-Verhalten lebender Blätter. Biochem. Zeits. **277**: 250–260. 1935.
137. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. IV. Mitteilung: Der Einfluss des Sauerstoffs auf das Fluoreszenzverhalten lebender Blätter. Biochem. Zeits. **278**: 373–385. 1935.
138. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. V. Mitteilung: Beziehungun zwischen Blattfluoreszenz und Sauerstoffkonzentration. Biochem. Zeits. **284**: 412–436. 1936.
139. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. VI. Mitteilung: Photographische Registrierung und Auswertung der Fluoreszensintensität-Zeitkurven grünen Blätter. Biochem. Zeits. **290**: 248–260. 1937.
140. ———. Chlorophyllfluoreszenz und Kohlensäureassimilation. VII. Mitteilung: Die Abhängigkeit des Verlaufs der Fluoreszenzkurven grünen Blätter von Sauerstoffdruck. Biochem. Zeits. **291**: 285–311. 1937.
141. KETELAAR, J. A. A., AND HANSON, E. A. Elementary cell and space group of ethylchlorophyllide. Nature **140**: 196. 1937.
142. KEUFFEL, C. W. A direct reading spectrophotometer. Jour. Opt. Soc. Am. **11**: 403–410. 1925.
143. KLEIN, G. Handbuch der Pflanzenanalyse III' 2 Spezielle Analyse II. 1932.
144. KNORR, H. V., AND ALBERS, V. M. Fluorescence of solution of chlorophyll *a*. Phys. Rev. **43**: 379. 1933.
145. ———, AND ———. Fluorescence and photodecomposition in solutions of chlorophyll *b*. Phys. Rev. **46**: 336. 1934.
146. ———, AND ———. Fluorescence of the chlorophyll series: Fluorescence and photodecomposition of solutions of chlorophyll *a* under O_2, CO_2 and N_2. Phys. Rev. **49**: 420. 1936.
147. KOEHN, C. J., AND SHERMAN, W. C. The determination of vitamin A and carotene with the photoelectric colorimeter. Jour. Biol. Chem. **132**: 527–538. 1940.
148. KOHN, H. I. Number of chlorophyll molecules acting as an absorbing unit in photosynthesis. Nature **137**: 706–707. 1936.
149. KUHN, R. Preparation of isomeric carotenes and their biological effects. Chemistry at the Centenary (1931) Meeting of the British Association for the Advancement of Science. (1932) pp. 108–112.
150. ———, AND BROCKMANN, H. γ-Carotin (Über das Vitamin des Wachstums, IV. Mitteil.). Ber. **66**: 407–410. 1933.
151. ———, AND ———. Über Rhodo-xanthin, den Arillus-farbstoff der Eibe (*Taxus baccata*). Ber. **66**: 828–841. 1933.

152. ———, AND ———. Bestimmung von Carotinoiden. Zeits. Physiol. Chem. **206**: 41–64. 1932.
153. ———, AND GRUNDMANN, C. Über Krypto-xanthin, ein Xanthophyll der Formel $C_{40}H_{56}O$ (Über das Vitamin des Wachstums, V. Mitteil.). Ber. **66**: 1746–1750. 1933.
154. ———, AND ———. Über Rubixanthin, ein neues Xanthophyll der Formel $C_{46}H_{50}O$. Ber. **67**: 339–344. 1934.
155. ———, AND LEDERER, E. Taraxanthin, ein neues Xanthophyll mit 4 Sauerstoffatomen. Zeits. Physiol. Chemie **200**: 108–114. 1931.
156. ———, AND MORRIS, C. J. O. R. Synthese von Vitamin A. Ber. **70**: 853–858. 1937.
157. ———, AND SMAKULA, A. Spektrophotometrische Analyse des Eidotterfarbstoffes. Zeits. Physiol. Chemie **197**: 161–166. 1931.
158. ———, AND WINTERSTEIN, A. Viola-xanthin, das Xanthophyll des gelben Stiefmutterchens (*Viola tricolor*). (Über konjugierte Doppelbindungen, XVI). Ber. **64**: 326–332. 1931.
159. ———, ———, AND LEDERER, E. Zur Kenntnis der. Xanthophylle. Zeits. Physiol. Chemie **197**: 141–160. 1931.
160. LEDERER, E., AND RATHMANN, F. H. A physico-chemical and biochemical study of vitamin A_2. Biochem. Jour. **32**: 1252–1261. 1938.
161. LEWIS, G. N., AND CALVIN, M. The color of organic substances. Chem. Rev. **25**: 273–328. 1939.
162. LEWKOWITSCH, E. The ultra-violet absorption spectrum of chlorophyll in alcoholic solution. Biochem. Jour. **22**: 777–778. 1928.
163. LUBIMENKO, V. N., AND GORTIKOWA, N. N. Über die Rolle des Sauerstoffs im Ergrünungsprosess. Beitr. Biol. Pflanz. **22**: 235–272. 1934.
164. ———, AND HUBBENET, E. R. The influence of temperature on the rate of accumulation of chlorophyll in etiolated seedlings. New Phytol. **31**: 26–57. 1932.
165. LUXTROL CO., Inc. New York. Photoelectric colorimeter.
166. MACKINNEY, G. On the crystal structure of carotenoids. Jour. Am. Chem. Soc. **56**: 488. 1934.
167. ———. Leaf carotenes. Jour. Biol. Chem. **111**: 75–84. 1935.
168. ———. On the plastid pigments of marsh dodder. Jour. Biol. Chem. **112**: 421–424. 1935.
169. ———. Some absorption spectra of leaf extracts. Pl. Physiol. **13**: 123–140. 1938.
170. ———. Applicability of Kundt's rule to chlorophyll. Pl. Physiol. **13**: 427–430. 1938.
171. ———. Kundt's rule. Pl. Physiol. **15**: 359. 1940.
172. ———. Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. **132**: 91–109. 1940.
173. ———. Plant pigments. Ann. Rev. Biochem. **9**: 459–486. 1940.
174. ———, AND MILNER, H. W. Carrot leaf carotene. Jour. Am. Chem. Soc. **55**: 4728–4729. 1933.
175. MANNING, W. M. Photosynthesis. Jour. Phys. Chem. **42**: 815–852. 1938.
176. MATLACK, M. B. Pigments of pink grapefruits, *Citrus grandis* (L, Osbeck). Jour. Biol. Chem. **110**: 249–253. 1935.
177. ———, AND SANDO, C. E. A contribution to the chemistry of tomato pigments. The coloring matter in American red and purple tomatoes (*Lycopersicum esculentum*). Jour. Biol. Chem. **104**: 407–414. 1934.
178. MCALISTER, E. D. The chlorophyll-carbon dioxide ratio during photosynthesis. Jour. Gen. Physiol. **22**: 613–637. 1939.
179. MCNICHOLAS, H. J. The visible and ultra-violet absorption spectra of carotin and xanthophyll and the changes accompanying oxidation. Bur. Stand. Jour. Res. **7**: 171–193. 1931.

180. MEAD, T. H., UNDERHILL, S. W. F., AND COWARD, K. H. Crystalline esters of vitamin A. I. Preparation and properties. II. Biological potency. Biochem. Jour. **33**: 589–600. 1939.
181. MELLON, M. G. The role of spectrophotometry in colorimetry. Ind. Eng. Chem., Anal. Ed. **9**: 51–56. 1937.
182. MENKE, W. Untersuchungen über das Protoplasma grüner Pflanzenzellen. II. Der Chlorophyllgehalt der Chloroplasten aus Spinatblättern. Zeits. Physiol. Chemie **263**: 100–103. 1940.
183. MEYER, K. P. Spektrometrische Untersuchungen über den Zustand des Chlorophylls in der Pflanze, in Extrakten und Reinpräparaten. Helv. Phys. Acta **12**: 349–393. 1939.
184. MICHAEL, G. Über die Beziehungen zwischen Chlorophyll- und Eiweissabbau im vergilbenden Laubblatt von *Tropaeolum*. Zeits. Bot. **29**: 385–424. 1935.
185. MILLER, E. S. Absorption spectra of alpha and beta carotenes and leaf xanthophyll at room and liquid nitrogen temperatures. Pl. Physiol. **9**: 179. 1934.
186. ———. Application of quantitative spectral analyses to binary mixtures of the common carotenoids. Pl. Physiol. **9**: 681–684. 1934.
187. ———. A rapid and accurate quantitative method for the determination of the common carotenoids; analyses of beta-carotene and leaf xanthophyll in thirteen plant tissues. Jour. Am. Chem. Soc. **57**: 347–349. 1935.
188. ———. Improved methods for the purification of the common carotenoids, and the quantitative measurement of their absorption spectra. Bot. Gaz. **96**: 447–467. 1935.
189. ———. A precise method, with detailed calibration for the determination of absorption coefficients; the quantitative measurement of the visible and ultraviolet absorption spectra of alpha carotene, beta carotene, and lycopene. Pl. Physiol. **12**: 667–684. 1937.
190. ———. Photoelectric spectrophotometry applied to the quantitative analyses of carotenoid and chlorophyll pigments in ternary and quaternary systems. Cereal Chemistry **15**: 310–316. 1938.
191. ———. Quantitative biological spectroscopy. 1939.
192. ———, AND JOHNSON, I. J. The relation between leaf tissue pigment concentration and yield in corn. Jour. Amer. Soc. Agron. **30**: 941–946. 1938.
193. ———, AND ———. Inheritance of chlorophyll in F_1 crosses made reciprocally between selfed lines of corn. Proc. Soc. Exp. Biol. & Med. **44**: 26–28. 1940.
194. ———, MACKINNEY, G., AND ZSCHEILE, F. P. Jr. Absorption spectra of alpha and beta carotenes and lycopene. Pl. Physiol. **10**: 375–381. 1935.
195. MONTFORT, C. Farbe und Stoffgewinn im Meer. Jahrb. Wiss. Bot. **79**: 493–592. 1934.
196. MÜLLER, R. H. Photoelectric methods in analytical chemistry. Ind. Eng. Chem., Anal. Ed. **11**: 1–17. 1939.
197. MULLIKEN, R. S. Intensities of electronic transitions in molecular spectra. IV. Cyclic dienes and hyperconjugation. Jour. Chem. Phys. **7**: 339–352. 1939.
198. ———. Intensities of electronic transitions in molecular spectra. VII. Conjugated polyenes and carotenoids. Jour. Chem. Phys. **7**: 364–373. 1939.
199. MUNSEY, V. E. Application of the neutral wedge photometer to the measurement of carotenoid pigments in flour and macaroni products. Jour. Assoc. Off. Agr. Chem. **21**: 331–351. 1938.
200. MYERS, J. A study of the pigments produced in darkness by certain green algae. Pl. Physiol. **15**: 575–588. 1940.
201. NICOLAI, M. F. E., AND WEURMAN, C. Some properties of chlorophyll-multifilms. Proc. Kon. Ak. Amst. **41**: 904–908. 1938.

202. NOACK, K. Der Zustand des Chlorophylls in der lebenden Pflanze. Biochem. Zeits. **183**: 135–152. 1927.
203. ———, AND KIESSLING, W. Zur Entstehung des Chlorophylls und seiner Beziehung zum Blutfarbstoff. Zeits. Physiol. Chemie **182**: 13–49. 1929.
204. ———, AND ———. Zur Kenntnis der Chlorophyllbildung. Zeits. Ang. Chemie **44**: 93–96. 1931.
205. OLTMAN, R. E. A new method and instrument for the quantitative determination of chlorophyll. Pl. Physiol. **8**: 321–326. 1933.
206. PADOA, M., AND VITA, N. Über die Wirkung von Kohlenoxyd auf frische Pflanzen. Untersuchungen über die Absorptionsspektra der Chlorophylle *a* und *b* in Gegenwart von CO, N_2, O_2, CO_2. Biochem. Zeits. **244**: 296–302. 1932.
207. PAULING, L. The significance of resonance to the nature of the chemical bond and the structure of molecules. Organic Chemistry: pp. 1850–1890. Gilman, H.
208. ———. The nature of the chemical bond. 1939.
209. PETERING, H. G., WOLMAN, W., AND HIBBARD, R. P. Determination of chlorophyll and carotene in plant tissue. Ind. Eng. Chem., Anal. Ed. **12**: 148–151. 1940.
210. PETERSON, P. D. Methods for the quantitative extraction and separation of the plastid pigments of tobacco. Pl. Physiol. **5**: 257–261. 1930.
211. ———, AND MCKINNEY, H. H. The influence of four mosaic diseases on the plastid pigments and chlorophyllase in tobacco leaves. Phytopath. **28**: 329–342. 1938.
212. ———, W. J., HUGHES, J. S., AND FREEMAN, H. F. Determination of carotene in forage. Ind. Eng. Chem., Anal. Ed. **9**: 71–72. 1937.
213. ———, ———, AND PAYNE, L. F. The carotenoid pigments. Occurrence, properties, methods of determination, and metabolism by the hen. Tech. Bull. 46. Kan. Agr. Exp. Sta. 1939.
214. PFALTZ AND BAUER, INC. New York. Photoelectric colorimeter, after Lange.
215. PHOTOVOLT CORP. New York. Lumetron photoelectric colorimeter.
216. ———. Small-spot photometer.
217. PORRET, D., AND RABINOWITCH, E. Reversible bleaching of chlorophyll. Nature **140**: 321–322. 1937.
218. PRINS, J. A. Spectrum of chlorophyll. Nature **134**: 457. 1934.
219. QUACKENBUSH, F. W., STEENBOCK, H. AND PETERSON, W. H. The effect of acids on carotenoids. Jour. Am. Chem. Soc. **60**: 2937–2941. 1939.
220. RABINOWITCH, E., AND WEISS, J. Reversible oxidation of chlorophyll. Proc. Roy. Soc. (London) A, **162**: 251–267. 1937.
221. RANDOLPH, L. F., AND HAND, D. B. Relation between carotenoid content and number of genes per cell in diploid and tetraploid corn. Jour. Agr. Res. **60**: 51–64. 1940.
222. RUDOLPH, H. Über die Einwirkung des farbigen Lichtes auf die Entstehung der Chloroplasten Farbstoffe. Planta **21**: 104–155. 1933.
223. RUSSELL, W. C., TAYLOR, M. W., AND CHICHESTER, D. F. The effect of the curing process upon the carotene and vitamin A content of alfalfa. N. J. Agr. Expt. Sta. Bull. 560: 1–8. 1932.
224. SARGENT, M. C. Causes of color change in blue-green algae. Proc. Nat. Acad. Sci. **20**: 251–254. 1934.
225. ———. Effect of light intensity on the development of the photosynthetic mechanism. Pl. Physiol. **15**: 275–290. 1940.
226. SCHARFNAGEL, W. Biologische Untersuchungen zur Chlorophyllbildung. Planta **13**: 716–744. 1931.
227. SCHERTZ, F. M. The quantitative determination of carotin by means

of the spectrophotometer and the colorimeter. Jour. Agr. Res. **26**: 383–400. 1923.
228. ———. The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **30**: 253–261. 1925.
229. ———. Some physical and chemical properties of carotene and the preparation of the pure pigment. Jour. Agr. Res. **30**: 469–474. 1925.
230. ———. Some physical and chemical properties of xanthophyll and the preparation of the pure pigment. Jour. Agr. Res. **30**: 575–585. 1925.
231. ———. The quantitative determination of chlorophyll. Pl. Physiol. **3**: 323–334. 1928.
232. ———. The extraction and separation of chlorophyll ($\alpha+\beta$) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Pl. Physiol. **3**: 211–216. 1928.
233. ———. The preparation of chlorophyll. Pl. Physiol. **3**: 487–497. 1928.
234. Scheunert, A., and Wagner, K. H. Untersuchung von Aphanin und Aphanicin auf Vitamin A—Wirkung. Zeits. Physiol. Chemie **260**: 272–275. 1939.
235. Seybold, A. Über die Lichtenergiebilanz submerser Wasserpflanzen, vornehmlich der Meeresalgen. Jahrb. Wiss. Bot. **79**: 593–654. 1934.
236. ———. Zur Kenntnis des Protochlorophylls. Planta **26**: 712–718. 1937.
237. ———, and Egle, K. Lichtfeld und Blattfarbstoffe. I. Planta **26**: 491–515. 1937.
238. ———, and ———. Lichtfeld und Blattfarbstoffe. II. Planta **28**: 87–123. 1938.
239. ———, and ———. Zur chromatographischen Methode der Blattpigmente. Planta **29**: 114–118. 1938.
240. ———, and ———. Zur Kenntnis des Protochlorophylls. II. Planta **29**: 119–129. 1938.
241. ———, and ———. Zur Kenntnis des Bakteriochlorophylls. Sitzungsb. Heidelb. Akad. Wiss. **1**: 7–17. 1939.
242. Sherman, W. C. Chromatographic identification and biological evaluation of carotene from mature soybeans. Food Res. **5**: 13–22. 1940.
243. ———, and Salmon, W. D. Carotene content of different varieties of green and mature soybeans and cowpeas. Food Res. **4**: 371–380. 1939.
244. Shrewsbury, C. L., and Kraybill, H. R. The carotene content, vitamin A potency, and antioxidants of butter fat. Jour. Biol. Chem. **101**: 701–709. 1933.
245. ———, ———, and Withrow, R. B. Determination of alpha- and beta-carotenes. Ind. Eng. Chem. Anal. Ed. **10**: 253–256. 1938.
246. Singh, B. N., and Rao, N. K. A. A new method for the detection of carotenoids in chlorophyll samples. Protoplasma **30**: 101–103. 1938.
247. Sjøberg, J. Beitrag zur Kenntnis der Bildung des Chlorophylls und der gelben Pflanzenpigmente. Biochem. Zeits. **240**: 156–186. 1931.
248. Smakula, A. Über physikalische Methoden im chemischen Laboratorium. XXII. Lichtabsorption und chemische Konstitution. Zeits. Ang. Chemie **47**: 657–665. 1934.
249. Smith, E. Solutions of chlorophyll-protein compounds (phyllochlorins) extracted from spinach. Science **88**: 170–171. 1938.
250. Smith, J. H. C. Carotene. III. Hydrogenation and optical properties of carotene and its hydrogenated derivatives. Jour. Biol. Chem. **90**: 597–605. 1931.
251. ———. The yellow pigments of green leaves: their chemical consti-

tution and possible function in photosynthesis. Cont. Marine Biology. Stanford Univ. Press. 1930.
252. ————. Carotene. X. A comparison of absorption spectra measurements on α-carotene, β-carotene and lycopene. Jour. Am. Chem. Soc. **58**: 247–255. 1936.
253. ————, AND MILNER, H. W. Carotene. VII. Physical properties of carotene from different plant sources. Jour. Biol. Chem. **104**: 437–447. 1934.
254. SMITH, L. L. W., AND MORGAN, A. F. The effect of light upon the vitamin A activity and the carotenoid content of fruits. Jour. Biol. Chem. **101**: 43–54. 1933.
255. SMITH, O. Effects of light on carotenoid formation in tomato fruits. Cornell Univ. Agr. Expt. Sta. Mem. 187: 3–26. 1935.
256. SPOEHR, H. A., SMITH, J. H. C., STRAIN, H. H., AND MILNER, H. W. Ann. Rep. Div. Pl. Bio., Carnegie Inst. Wash. 1935, 1936, 1937.
257. SPRAGUE, H. B. A convenient method of measuring quantities of chloroplast pigments. Science **67**: 167–169. 1928.
258. ————, AND CURTIS, N. Chlorophyll content as an index of the productive capacity of selfed lines of corn and their hybrids. Jour. Amer. Soc. Agron. **25**: 709–723. 1933.
259. ————, AND SHIVE, J. W. A study of the relations between chloroplast pigments and dry weight of tops in dent corn. Pl. Physiol. **4**: 165–192. 1929.
260. ————, AND TROXLER, L. B. An improved color standard for the colorimetric determination of chlorophyll. Science **71**: 666–667. 1930.
261. STAIR, R., AND COBLENTZ, W. W. Infrared absorption spectra of some plant pigments. Bur. Stand. Jour. Res. **11**: 703–711. 1933.
262. STAUDIGER, H., AND STEINHOFER, A. Über hochpolymere Verbindungen, 105. Mitteil: Viskositätsmessungen an Carotinoiden. Ber. **68**: 471–473. 1935.
263. STEELE, C. C. Recent progress in determining the chemical structure of chlorophyll. Chem. Rev. **20**: 1–37. 1937.
264. STERN, A., ET AL. Über die Lichtabsorption der Porphyrine. I. Zeits. Physik. Chemie (A) **170**: 337–350. 1934.
265. ————. Über die Lichtabsorption der Porphyrine. II. Zeits. Physik. Chemie (A) **174**: 81–103. 1935.
266. ————. Über die Lichtabsorption der Porphyrine. III. Zeits. Physik. Chemie (A) **174**: 321–334. 1935.
267. ————. Über die Lichtabsorption der Porphyrine. IV. Zeits. Physik. Chemie (A) **175**: 405–437. 1936.
268. ————. Über die Lichtabsorption der Porphyrine. V. Zeits. Physik. Chemie (A) **176**: 81–124. 1936.
269. ————. Über die Lichtabsorption der Porphyrine. VI. Zeits. Physik. Chemie (A) **177**: 40–81. 1936.
270. ————. Über die Lichtabsorption der Porphyrine. VII. Zeits. Physik. Chemie (A) **177**: 165–192. 1936.
271. ————. Über die Lichtabsorption der Porphyrine. VIII. Zeits. Physik. Chemie (A) **177**: 365–386. 1936.
272. ————. Über die Lichtabsorption der Porphyrine. IX. Zeits. Physik. Chemie (A) **177**: 387–397. 1936.
273. ————. Über die Lichtabsorption der Porphyrine. X. Zeits. Physik. Chemie (A) **178**: 161–183. 1937.
274. ————. Über die Lichtabsorption der Porphyrine. XI. Zeits. Physik. Chemie (A) **179**: 275–294. 1937.
275. ————. Über die Lichtabsorption der Porphyrine. XII. Zeits. Physik. Chemie (A) **180**: 131–138. 1937.
276. ————. Über die Absorptionsspektren der Pyrrolfarbstoffe. I. Zeits. Physik. Chemie (A) **180**: 25–43. 1937.

277. ———. Über die Absorptionsspektren der Pyrrolfarbstoffe. II. Zeits. Physik. Chemie (A) **182**: 117–126. 1938.
278. ———. Zur Fluorescenz der Porphyrine. I. Zeits. Physik. Chemie (A) **175**: 38–62. 1935.
279. ———. Zur Fluorescenz der Porphyrine. II. Zeits. Physik. Chemie (A) **176**: 209–225. 1936.
280. ———. Zur Fluorescenz der Porphyrine. III. Zeits. Physik. Chemie (A) **176**: 347–357. 1936.
281. ———. Zur Fluorescenz der Chlorine. Zeits. Physik. Chemie (A) **182**: 186–192. 1938.
282. ———, AND PRUCKNER, F. Zur Lichtabsorption der Imido-porphyrine. Zeits. Physik. Chemie (A) **178**: 420–436. 1937.
283. ———, AND ———. Lichtabsorption und Konstitution einiger Derivate der Chlorophylle. Zeits. Physik. Chemie (A) **180**: 321–358. 1937.
284. STOLL, A. Zusammenhänge zwischen der Chemie des Chlorophylls und seiner Funktion in der Photosynthese. Naturwiss. **24**: 1–16. 1936.
285. ———, AND WIEDEMANN, E. Die optische Aktivität des Chlorophylls. Helv. Chimie. Acta **16**: 307–314. 1933.
286. ———, AND ———. Chlorophyll. Forts. Chemie Organ. Nat. I: 159–254. 1938.
287. STRAIN, H. H. Carotene. VIII. Separation of carotenes by adsorption. Jour. Biol. Chem. **105**: 523–535. 1934.
288. ———. Carotene. IX. Carotenes from different sources and some properties of α- and β-carotene. Jour. Biol. Chem. **111**: 85–93. 1935.
289. ———. Formation of carotenoids and chlorophylls in etiolated barley seedlings exposed to red light. Pl. Physiol. **13**: 413–418. 1938.
290. ———. Leaf xanthophylls. Carnegie Inst. Wash. 1938.
291. STROTT, A. Der Einfluss der Umweltbedingungen auf die Ausbildung der Chloroplastenfarbstoffe. Jahr. Wiss. Bot. **86**: 1–32. 1938.
292. SUMNER, F. B., AND FOX, D. L. Studies of carotenoid pigments in fishes. II. Investigations of the effects of colored backgrounds and of ingested carotenoids on the xanthophyll content of *Girella nigricans*. Jour. Exp. Zool. **71**: 101–123. 1935.
293. TAYLOR, W. H. An X-ray examination of β-carotene. Zeits. Kristallogr. **96**: 150–154. 1937.
294. TILDEN, J. E. A classification of the algae based on evolutionary development, with special reference to pigmentation. Bot. Gaz. **95**: 59–77. 1933.
295. TOTTINGHAM, W. E., AND DUTTON, H. J. The effect of spectral regions on the chlorophyll "A" to "B" ratio. Science **87**: 214. 1938.
296. TSWETT, M. Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. Ber. Deut. Bot. Ges. **24**: 384–393. 1906.
297. VAN NIEL, C. B., AND ARNOLD, W. The quantitative estimation of bacterio-chlorophyll. Enzymol. **5**: 244–250. 1938.
298. ———, AND SMITH, JAMES H. C. Studies on the pigments of the purple bacteria. I. Arch. Mikrobiol. **6**: 219–229. 1935.
299. VON BERNEGG, A. S., HEIERLE, E., AND ALMASY, F. Spektrophotometrische Bestimmung von Chlorophyll *a*, Chlorophyll *b*, Carotin and Xanthophyll. Biochem. Zeits. **283**: 45–52. 1935.
300. WAKKIE, J. G. Notes on the possible structure of the chlorophyll-granules in the plastid. Proc. Kon. Ak. Amst. **38**: 1082–1086. 1935.
301. WASSINK, E. C., ET AL. On the fluorescence of photosynthesizing cells. Enzymol. **4**: 254–268. 1937.
302. ———. On the relation between fluorescence and assimilation in photosynthesizing cells. Enzymol. **5**: 100–109. 1938.

303. ———. Theoretical considerations concerning the relation between chlorophyll fluorescence and photosynthesis in green plant cells. Enzymol. **5**: 110–118. 1938.
304. ———. The initial changes of chlorophyll-fluorescence in *Chlorella*. Enzymol. **6**: 145–172. 1939.
305. ———. Infrared absorption spectra of various strains of purple bacteria. Enzymol. **7**: 113–129. 1939.
306. WATKINS, W. E. Monthly variation in carotene content of two important range grasses, *Sporobolus flexuosus* and *Bouteloua eriopoda*. Jour. Agr. Res. **58**: 695–699. 1939.
307. WEIER, E. The structure of the chloroplast. Bot. Rev. **4**: 497–530. 1938.
308. WILKENS-ANDERSON Co. Chicago, Ill. KWSZ photometer.
309. WILSON, J. N. A theory of chromatography. Jour. Am. Chem. Soc. **62**: 1583–1591. 1940.
310. WILLSTÄTTER, R., AND STOLL, A. Investigations on chlorophyll. Methods and results. Translated by Schertz, F. M. and Merz, A. R. The Science Press Printing Co. Lancaster, Pa. 1928.
311. WINTERSTEIN, A. Fraktionierung und Reindarstellung von Pflanzenstoffen nach dem Prinzip der chromatographischen Adsorptionsanalyse. (g) Chlorophylle. Handb. Pflanzenanalyse **4**: 1426–1432. 1933.
312. ———. Über ein Vorkommen von γ-carotin. Zeits. Physiol. Chemie **219**: 249–252. 1933.
313. ———, AND SCHÖN, K. Fraktionierung und Reindarstellung organischer Substanzen nach dem Prinzip der chromatographischen Adsorptionsanalyse. III. Mitteilung: Gibt es ein Chlorophyll *c?* Zeits. Physiol. Chemie **230**: 139–145. 1934.
314. ———, AND STEIN, G. Fraktionierung und Reindarstellung organischer Substanzen nach dem Prinzip der chromatographischen Adsorptionsanalyse. II. Mitteilung: Chlorophylle. Zeits. Physiol. Chemie **220**: 263–277. 1933.
315. WISEMAN, H. G., KANE, E. A., SHINN, L. A., AND CARY, C. A. The carotene content of market hays and corn silage. Jour. Agr. Res. **57**: 635–669. 1938.
316. ZECHMEISTER, L. Carotinoide. 1934.
317. ———, AND CHOLNOKY, L. Die chromatographische Adsorptionsmethode. 1937.
318. ———, ———, AND POLGAR, A. Über die Isomerisierung des Zeaxanthins und Physaliens. Ber. **72**: 1678–1685. 1939.
319. ———, AND ———. Zur Isomerisierung von Xanthophyllen. Ber. **72**: 2039–2041. 1939.
320. ———, AND TUZSON, P. Isomerization of carotenoids. Biochem. Jour. **32**: 1305–1311. 1938.
321. ———, AND ———. Umkehrbare Isomerisierung von Carotinoiden durch Jod-Katalyse. Ber. **72**: 1340–1346. 1939.
322. ZIMMERMAN, W. I., TRESSLER, D. K., AND MAYNARD, L. A. Determination of carotene in fresh and frozen vegetables. I. Carotene content of green snap beans and sweet corn. Food Res. **5**: 93–101. 1940.
323. ZSCHEILE, F. P. An improved method for the purification of chlorophylls A and B; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. Bot. Gaz. **95**: 529–562. 1934.
324. ———. A quantitative spectro-photoelectric analytical method applied to solutions of chlorophylls a and b. Jour. Physic. Chem. **38**: 95–102. 1934.
325. ———. Investigation of the fluorescence spectra of chlorophylls A and B in ether solution. Protoplasma **22**: 513–517. 1935.
326. ———. Toward a more quantitative photochemical study of the plant cell's photosynthetic system. Cold Spring Harbor Symposia Quant. Bio. III: 108–116. 1935.

327. ———. Unpublished results. 1936.
328. ———, AND COMAR, C. L. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. Bot. Gaz. **102**: 463–481. 1941.
329. ———, HOGNESS, T. R., AND YOUNG, T. F. The precision and accuracy of a photoelectric method for comparison of the low light intensities involved in measurement of absorption and fluorescence spectra. Jour. Physical Chem. **38**: 1–11. 1934.
330. ———, AND WHITE, J. W. JR. Microscope hot stage for determination of melting points. Application to carotenoid pigments. Ind. Eng. Chem., Anal. Ed. **12**: 436–438. 1940.

THE BOTANICAL REVIEW

VOL. VII DECEMBER, 1941 No. 12

GROWTH RINGS AND CLIMATE

WALDO S. GLOCK
Texas Technological College

INTRODUCTION

The literature of the nineteenth century abounds with references directly to annual layers or indirectly to the ages of various trees. Henry Thoreau "was an impassioned measurer and gauger, and he caught his death in a way as significant as that of Francis Bacon, while counting the growth rings on felled timber" (189). The editor (180) of the *American Journal of Science* thought it worth his while to record the discovery of the age of a great oak felled in Poland in 1812. Indeed, the lore of "tree rings"—for without doubt they have been popularly called "rings" ever since they were first seen on stump or windfall—goes far back in the writings of man.

Interpretations, too, began in an early day. Leonardo, more than 400 years ago (141, 146), pointed out that the age of a tree may be determined by counting its rings, that water has great importance for tree growth, and that the thickness of the rings reveals the nature of the seasons, whether wet or dry. "He explained to Francesco how to arrive at the years of a split tree trunk by the number of its rings; and, by the thickness of these rings, at the degree of moisture of the corresponding year; . . . ." In his "Voyage en Italie," Michel Montaigne, says von Humboldt (108), was the first to notice the relation of annual rings to the age of a tree. A skillful artist had, in turn, called Montaigne's attention to the age relation, at the same time maintaining that rings habitually are narrower on the north side of a tree. Later research brought out the failure of the quarter of the heavens as a determinant of annual deposits. In one of his charming books, Holmes (107) reads the life story of a fallen hemlock in the variable thicknesses of its rings: how it grew rapidly, then slowly, then uniformly until the sluggishness of age overtook it. The section of wood resembled a "wooden preacher" telling of good conditions and of bad.

The work of J. Kuechler (75, 76, 117, 123) merits a word because he was one of the first to exercise a high degree of selection in the choice of his trees and because his botanical assumptions and meteorological inferences came in for rather severe criticism at the hands of Cleveland Abbe (1). Originally, Kuechler's results were published in 1859 in the "Zeitung" of San Antonio, Texas. "Mr. Kuechler seems to have adopted the idea that a tree bears the history of its climatic surroundings written in itself, and that its annual rings of growth vary in size mainly with the supply of water to the roots, so that broad rings indicate wet years and thin rings that can scarcely be distinguished with the naked eye denote dry years." He studied 3 post-oaks taken from "high isolated positions so that the drought should have an early effect." Thicknesses of the rings from the 3 trees corresponded exactly, thus "confirming the idea that moisture is the principal cause of the differences in the breadth of rings." Based on the belief that each ring represents a year and that each ring faithfully records the rainfall of its season, Kuechler arranged his rings and hence the years in a series from very wet to very dry.

In regard to the inferences of Kuechler, Abbe (1) makes the following points: *a*) "In Texas two quite distinct periods of growth and repose" have been observed "within one year." It is of interest that at about the same time in Arizona Furras (73) cites abundant proof that rings are not annual; and thus what the paper calls another scientific axiom is laid to rest. *b*) "The large number of very wet years . . . is not at all in accord with the rainfall records during the years 1840 to 1890, and, in fact, no region on the globe is known where the distribution of the rainfall is similar to that given by these records." *c*) "The tree growth can not be adopted as an index of the rainfall alone unless it is proved by the biologist that rainfall alone affects the growth, which is well known to be far from true. The annual rings certainly depend at least in part upon the evaporation, the sunshine, the temperature, and the distribution of rain in frequent showers or in frequent heavy floods." *d*) Much growth simply means favorable conditions and slight growth unfavorable. In truth, the inferences of Kuechler and the objections of Abbe almost duplicate the situation of today.

One of the best reviews of early work in Europe has been made by Erlandsson (66). Wastenson (66*f*) says Linné himself was the

first to relate tree rings to climate by ascribing (66*b*) the size of oak rings to the severity of winters. In contrast, Broocman (66*a*) in 1736 emphasized the effect of favorable summer weather on the width of annual rings. Rudenschöld (66*e*) noted the difference in growth between north and south Finland and emphasized the importance of climate, soil and intercongruence of the trees. In the early part of the nineteenth century, Reventlow (66*d*), according to Erlandsson, made the first measurements of rings. And then in 1869, be it noted, Pokorny (66*c*) explained a method for obtaining meteorological values from the annual growth of trees.

A brief introductory survey of concepts is not complete without reference to experimental work such as that of von Mohl, Knight, Nördlinger, Metzger, R. Hartig and F. Schwarz, described by Haberlandt (94). Knight, who fixed the trunk of an apple tree to bend only in one plane, found that greatest growth took place in the direction of bending. Differences in the general conditions of nutrition in successive seasons cause variations in the thickness of successive annual rings in any one transverse section. For instance, insect ravages give a narrow ring, and a season favorable to development of foliage gives a wide ring. "In this way the fluctuations in the width of the successive annual rings in the trunk form a permanent record of the principal episodes in the history of the tree." Haberlandt also stresses the work of von Mohl and Schwarz on the variations in thickness longitudinally in the trunk showing a tendency to form a "girder of uniform strength."

Actually, dozens of references could be cited to early work on growth layers but they would quickly become repetitious and wearisome. All in all, the publications contain a curious mixture of inference and interpretation, and here and there a thread of sound botanical investigation. It is even so today, for temptations to short-cut are numerous and attractive.

GROWTH FACTORS AND THEIR COMPLEXITY

Tree growth summates the physiological success of a tree in coping with conditions of its environment and using the materials thereof. Although water has been said (135) to be the most important constituent of the environment, growth actually depends upon the "entire constellation" (177) of conditions, and rarely is there "justification for attributing inequalities of growth under natural

conditions to the fluctuations of a single environmental condition." Their complexities are such that the reactions of one factor must always be considered in the light of all the others. Even though one factor appears to be paramount, this apparent simplicity (162) may actually be underlain by startling complexity.

The relation between a plant and its environment (33, 177) is indeed complex. In fact, "The environment of any organism consists of everything in the universe external to the cells and intimately utilized cell products of that particular organism" (4). "No one factor taken alone and divorced from all other contributing conditions, such as light, temperature, moisture, minerals, *etc.*, can be considered as significant in the life of plants" (22). The fundamental complexity, the simultaneous interaction and mutual dependency are so well known as to require no further emphasis.

Botanical literature abounds with lists of factors. For instance, Meyer and Anderson (147) give a rather exhaustive list of habitat factors as botanists understand them, whereas Toumey and Korstian (191) enumerate site factors as foresters see them. Such arrays impress the serious worker with the problems encountered in a study of the relationship between a tree and its habitat. Small wonder that conjectures and speculation have been said (23, 168) to replace long arduous work, at times. In contrast, the methods of Pearson (152) and of Adams (3) are especially directive, among the many excellent examples of detailed investigations of several factors at a time.

Almost without exception every student is conscious of the many factors having an influence upon tree growth. Attempts have been made (3, 9, 14, 18, 37, 46, 78, 122, 131, 166, 168) either to consider all factors or to treat several while the rest are held in mind. Great difficulties attend such efforts, and the reader is impressed, as the student himself must have been, by the complex nature of the interactions among biologic and among site factors. Several workers (9, 38, 46, 116, 173, 174) insist that the multiple action of factors can not be neglected, and try by all the means available to test the tree growth-rainfall relationship. Kleine, Potzger and Friesner (119) say that other factors do not have such a range of variation as do precipitation and temperature which may therefore be expected to record their effects in tree growth. This is denied in part by Fry and White (71).

According to others (58, 82, 97, 121, 125, 129, 163), rainfall or rainfall and temperature are the dominant factors in semiarid regions. In 1935 Lyon (129) seemed to think the rôle of water as a limiting factor in arid regions had been proved; but in 1940 (134) a measure of doubt crept in, for he speaks of the masking effect of climatic factors other than rainfall, of the discrepancy between amount of rainfall and amount of water entering the soil, of the distribution of rainfall within the month, of intensity of rainfall, and of the relation of temperature to water and growth rate of meristematic tissues. Salisbury and Jane (163) recognize the great importance of water in dry regions while bearing in mind the effects of other factors. Interestingly enough, these students do not depend upon exact thicknesses of growth layers. They do emphasize and rely upon the general character of the annual increments and "the range of variation they exhibit in relation to those of other periods."

No one will deny the importance of water in semiarid regions unless experiment and observation should prove some other factor or combination of factors more likely to be recorded in growth. The work of Pearson (152) amply supports the vital rôle of water. However, water is just as important in humid regions as in semiarid, the difference being not so much in the critical need of moisture as in the rate at which the crises impinge upon the plant. Because of species differences a drought in a humid region may be as disastrous to trees as in a semiarid region, other factors being similar.

It is somewhat puzzling to understand in a few cases (32, 47, 51, 66, 82, 89, 102, 185) why the existence of many growth factors is recognized and yet one only, rainfall, is commonly selected for tree-growth comparisons. Undoubtedly, water has the highest importance to plants for purposes of nutrition, translocation, transpiration, turgor, and even the origin of plants (135). Does its significance, however, justify the expectation that the effects of rainfall alone shall be recorded in recognizable fashion in the trees? Furthermore, it is difficult, in view of the known complexity of weather variations on the earth, to accept Mrs. De Geer's (47) apparent belief that so far as growth factors are concerned the earth is essentially a unit.

Space will not permit more than a list of factors, other than rainfall, which have been emphasized: release from competition (3, 69, 130, 174); release by fire (116); release by drainage (127); release by lumbering (10); interception of rainfall by the crown (3,

110); seed production (7); temperature (13, 13*a*, 13*e*, 78); details of rainfall distribution, intensity and disposal (18, 71, 159, 162, 179); food manufacture or reserves (13*a*, 13*c*, 38, 138, 145, 174); light (13*b*, 22, 182); defoliation (67*a*); fog (53); length of growing season (71); runoff (97); heredity or the fundamental property of variation in an organism (23, 106); leaf area (196); tornadoes (194); soil (122, 162, 164, 175); growth energy or aging (122, 175); cambium temperature (138); auxins or activators (65, 138); drought, high temperature and high wind (144); evaporation (13*d*); and soil moisture and its relations (152, 159).

A careful reading of the work having to do strictly with the comparison of width of growth layers and rainfall is apt to convey the impression that the fundamentals of plant anatomy and physiology either have been neglected or have not been investigated. The latter most certainly is not the case, for the references to basic studies are legion and far beyond the scope of our task. However, certain work must be mentioned because it applies directly and pertinently to growth-layer studies or because it constitutes a highly necessary background. The attempts to apply basic knowledge directly to growth layers are none too numerous (3, 9, 38, 46, 71, 116, 134, 173, 174, 183). Such attempts may well be summarized for us by Davis and Sampson (46) where they say their "study supports the much-expressed conclusion that interaction of plants to their environment is so complex that the existence of relationship of any one physical factor to plant-function is obscured." Even a few of the special studies appertaining to tree growth cover a wide range of topics: weather areas and their extensions (6); anatomical effects of artificial defoliation (42); distribution of rainfall in time (46, 104, 178); distribution of food materials and depletion of reserves (138*a*, 138*b*); local or microclimate (24, 110, 156, 157, 164, 179); chemical analyses of different types of wood in the growth layers (155*a*); effect of soil-moisture availability (115); hormones, vitamins and trace elements (65, 137); carbohydrate-nitrogen ratio (145); experimental work and the life history of a tree (20, 137, 138, 150, 154, 177); complex character of the soil as a reservoir (164); and the effects of insect attacks (67, 188). Jenny and Overstreet (112) make the interesting suggestion that the presence of a liquid solution may not be necessary for the absorption of mineral salts by a plant.

A choice of material basic to research on growth layers is extremely difficult to make. Certain studies, however, are fundamental (3, 23, 24, 33, 37, 40, 41, 74, 105, 106, 111, 128, 136, 137, 138, 139, 143, 147, 152, 153, 155, 158, 159, 162, 164, 168, 190, 195, 200, 203), and the opinion is ventured that a knowledge of them is with little doubt a necessary prerequisite to the adequate consideration of growth layers. Although far from complete, the above list does furnish a background of recent work which indicates our fast increasing awareness of basic factors and which will spare us the indictment that "Frequently the investigator fails to consider all the factors of the environment, securing data on those conditions in which he is particularly interested and neglecting or theorizing as to the others" (3). Experimental work on a controlled basis, such as by Burns (24), to give but one example, goes a long way to assure a final understanding of the relative importance of the many growth factors.

No serious objection is made to the statement of Lathe and McCallum (125): "The annual growth rings of trees constitute a permanent and reasonably accurate record of the reaction of the trees to their environmental conditions." Difficulties arise in attempts to isolate some one factor in the environment. Work by Liebig and Mitscherlich (147) on the law of the minimum and by Blackman (15) on limiting factors strongly suggests a decided control by one factor, but these studies, in addition to much of the material cited in this section, give no basis as yet for the assumption that tree growth is an exact measure of a single factor. So far, all studies, experimental, observational or statistical, simply emphasize what very few students will deny, the great importance of water to plant life. To go further than this at present is to lose sight of the fact that the deposition of cellulose is a complicated process (147, 168).

INSECTS

Pearson (153) has so concisely, so ably discussed various factors whose influence on growth may be confused one for the other that hesitation to add anything is excusable except in respect to insect attacks. The anatomical effects of such attacks, resulting in partial or complete defoliation, bear strong resemblance to drought influence. Artificial defoliation has been practiced (42, 137, 138) and the results studied. In the season of defoliation, MacDougal (138)

noted an increased production of wood; subsequently, there was a reduction accompanied by modification of structure. Craighead (42) noted a slight reduction in wood formation the first year and a more severe reduction the second and third years with repeated partial defoliations. Thus, the effects do not necessarily strike the trees instantly, as recorded by Pearson (153). In a summary of his work on defoliation, MacDougal (137) says: "the anatomical features described and illustrated incorporate in the trunk departures from normal structure which may make serious difficulties when the attempt is made to construe the layers as a record of climatic effects."

Insects cause partial or complete defoliation (5, 67, *67a*, *67b*, 125, 182) which can be sufficiently severe to kill trees or at least to prohibit the formation of wood in the lower portion of the trunk. Sperry (182) reports two attacks annually by a bark beetle in Colorado, the first in June–July, the other in August–September. Such attacks, if severe enough, can interfere with the "normal" development of a growth layer and must be taken into consideration by students of growth layers, especially if attacks are widespread and epidemic repeatedly in a region (67).

Indeed, the work of Evenden (67) indicates the great likelihood of mistaking insect attacks for drought. The record of impact and of recovery for insects or drought may be quite similar. Repeated attacks by the pine butterfly have occurred in Idaho, Washington and nearby Canada since 1882. The zones of diminished growth which were measured at the bases of the trees commonly failed to include a full number of growth layers for the true number of years covered. Such zones of attack and recovery vary in length from 2 or 3 years up to 10, 12 or more years. In addition, a zone simply of diminished growth might equally well be interpreted either as insect attack or as drought. Combine these facts with the widespread area over which an attack may occur, and the uncertainty of climatic interpretation because of one factor becomes quite apparent (116, 125). The probability must be recognized that what has been labelled as drought by Haury (99) may well have been something else (31, 32, 168).

CROSS-DATING

Under certain conditions and in certain localities the variations in the growth-layer sequence of one tree matches rather generally

the variations of the sequence in another tree. Such correlation has been called cross-dating (58, 82), and assumes that each sharply defined growth layer, complete or incomplete as a sheath of xylem, constitutes an annual increment. It is to be noted that the matching of sequences is done on a linear, not volume basis. Although the cross-dating of sequences in a portion of a stand whose trees grow under essentially similar conditions depends probably upon environmental response worthy of serious study, the specific causes of such correlation have not been investigated. Cross-dating is implied if more than one sequence is measured and the measurements averaged.

Kuechler (117) employed cross-dating as long ago as 1859 to confirm the response of trees to moisture. Long before him botanists may well have recognized the same rather general uniformity of variation among certain trees. A great many students in late years (9, 18, 46, 66, 77, 97, 99, 100, 116, 119, 126, 132, 163, 174, 183) tacitly accept or use cross-dating apparently without question. Even Oliver Wendell Holmes (107) speaks of a "calendar counted on the rings." In one instance (119), the years 1912–1917 on the wood serve as a check to eliminate dating errors. Evenden (67) uses a growth pattern of two years for purposes of correlation. These seem short; nevertheless, their recency combined with the nature of the problems give them a security which extension into past centuries may not have. Lathe and McCallum (125) unite the averages of many trees but do not mention cross-dating. Because of the narrowness of growth layers and because of their rather indefinite boundaries, Erlandsson (66) is none too sure of his correlation methods.

A few workers insist that cross-dating is fundamental, first, for the accuracy of year-to-year dating, and second, for the derivation of climatic history. In spite of long-continued research by botanists in physiology, anatomy and ecology, the belief among the few in the absolute accuracy of identification of annual growth layers has become increasingly insistent (52, 56, 58, 64, 82, 100, 185). Tangentially incomplete rings which had been considered at first to be only one portion of an annual increment were later proved to be annual, Douglass believes (64), by cross-dating. Merging of several sequences into an average builds a chronology in which "Each ring has its absolute calendrical date" (185). The implication from

the foregoing can not be evaded: the formation of a distinct growth layer is necessarily an annual phenomenon. Or, to put it somewhat differently, influential growth factors, held to be climatic, regulate cambial activity and derivative xylem in the trees of a locality on a yearly basis only. A drought, for instance, in the middle of the actual growing season can not cause a definite cessation of growth, or, if it does, growth can not be resumed until the following year. Furthermore, many students find difficulty in subscribing completely to the practice whose essence is embodied in the statement of Stallings (185): "The date of any . . . ring . . . is simply determined by allowing one year for each ring in the pattern. . . ." Cross-dating is held to make this possible. By the system of dating, the assignment of an exact year to a growth layer formed centuries ago is so rigid that, as Haury says (100), ". . . the quantity is unchanging; it is either right or wrong."

Next in importance to the assumption that cross-dating proves the annual identity of a growth layer, there is the one pertaining to climatic interpretations. Cross-dating is held to be a prerequisite to the use of growth layers as climatic indicators (53, 56, 58, 61, 64, 129, 185), and in some cases its use has been urged until it approaches what may be considered an ecologic extreme. "The accurate dating of rings gives a means of studying quantitatively climatic reactions in trees through a series of many years" (61). Although the logic is confused and difficult to follow, the supposed function of cross-dating may be detected in the words of Douglass (64): "The resulting careful comparison [*i.e.*, cross-dating] between different trees . . ., because these patterns are climatic, insures the climatic origin of the factors on which the cross-dating depends." If only those trees which cross-date carry a record of climate, then to what do the rest of the trees respond? It is to be noted in passing that many of those who speak of climate in connection with growth layers actually refer to rainfall, and that the relation to rainfall commonly depends upon statistical correlation.

Trees which do not cross-date might just as well be discarded because they do not yield a climatic record (58, 64, 82, 134). Until such belief is founded upon fact it must be examined in the light of present botanical knowledge. Adams (3) found that one group of trees increased its rate of diameter growth with thinning whereas another group decreased it. All trees are a product of

hereditary and environmental factors (106). Different trees may exhaust their available soil moisture at varying rates because of soil conditions about their roots and yet receive equal amounts of rainfall. The cross-dating of a certain proportion of the trees out of a group means that such trees have microclimates, microsoil conditions, competition, or one or more other factors roughly similar to each other. The rest of the trees are just as representative although they do not cross-date. As a matter of fact, cross-dated trees may exaggerate a local condition (110, 161) which is not representative of the area. Not all students, however, restrict themselves (116, 125, 167, 168) to just a few trees.

Salter's studies (164) on soil suggest that different soils around various trees under different amounts of rainfall may make available to the trees similar amounts of soil moisture; hence, a rough correspondence in tree growth. Potzger (156, 157) studied microclimate in relation to exposure, slope direction, evaporation and soil moisture in different locations. The differences he found between evaporation and soil moisture on a north slope and on a south slope raise the query: if the south slope trees should cross-date and the north slope trees do not, then do the south slope trees (161) alone yield an accurate record of rainfall? If we combine the variations of root systems (203) with the variations of soil (164) and with the variations of soil-water replenishment (178, 179), to mention but three, we are at once dealing with compensating factors (147) whose complicated interactions may be amply sufficient to give a rough correspondence in width of growth layers among trees now and then through the years. The practice of cross-dating impinges directly upon the high selectivity exercised in the choice of trees *after their samples are gotten to the laboratory.*

The complete picture of cross-dating also depends upon age counts, qualitative accuracy, significance of cross-dating, and basic botanical research. A section of a tree or a portion out of a group of trees may show too few or too many growth layers for the years involved (5*a*, 67, 88, 114, 137, 154, 192). As regards too few layers, cross-dating is sometimes of distinct assistance (64, 82) in locating where their absence occurs and in certain cases in determining how many layers are absent. It can not prove, however, either the annual or the intraseasonal identity of the layers concerned.

Because of innate variations among growth layers it is difficult to set a standard as to what constitutes qualitative accuracy of cross-dating. The problem has two aspects, the length of record and the number of correlated growth layers in the records. Douglass (58) and Glock (82) think 50 years sufficiently long, as a rule; but this depends entirely on how many growth layers in the compared sequences resemble each other and how certain the resemblance (8). Glock (82) considers 80% similarity as necessary for dependable cross-dating, leaving a 20% margin for inherent differences (83) among trees even though they grow close together. How uncertain and how tenuous cross-dating may become is apparent in the light of genetic and environmental variations and in view of the lack of a reliable standard (8) as to what constitutes cross-dating and what does not. How many layers out of each century *appear* to be similar in relative thickness on two sequences which are said to cross-date? Twenty to 25 per century is a very high average. Opinion, as well as practice, is bound to vary. Some will wonder how little resemblance constitutes acceptable cross-dating when they learn from Douglass that he (54) cross-dated coast redwoods "by means of occasional deficient rings" which gave a "sequence of excellent dating." In connection with coast redwoods numerous caution signals (69, 70, 137, 138) have been raised.

Goldthwait and Lyon (89) found the layers for certain years relatively wide or narrow; in other years the layers had much variation between individual trees. They considered 31 growth layers out of 63 to have relatively greater or less growth rates than the immediately preceding or following layers. Such agreement without visible demonstration of the matter seems very good. In contrast, Lyon (129) achieves his cross-dating by the comparison of two graphs, and considers the correspondence to be obvious. The interval, 1751–1780, was selected at random from his graphs and the trend taken; that is, the years were enumerated in which the growth in the two trees was parallel in direction, or was not. For the interval, 1751–1780, 12 years had parallel trend and 18 had opposite. Surely this can not be held as good cross-dating. A year later, Lyon (131) compared the graphs of 5 trees and found 14 cases in some 220 years of perfect agreement among the layers narrower or wider than their immediate neighbors. He analyzes the interval, 1801–1850, for the 5 trees and finds 12 perfect agree-

ments out of the 50, which he considers a significant correlation because the law of probability allows 1 out of 16. His arguments lose weight, however, when his list of narrow and wide layers for 1600–1934 is examined. He mentions a total of 118 years, and his "entries have been made only when essentially the same date was marked by a maximum or a minimum at two-thirds or more of the sites." The total of 118 consists of single entries arranged in groups. That is to say, the trees do not all have a narrow (or wide) layer on the same date. One will be a year late, another two years, and a third a year early. There seems to be far from perfection of cross-dating where the diagnostic rings occur over a range of three or four years.

Keen (116), working with 44 groups scattered over eastern Oregon and northeastern California, uses key years to carry his cross-dating back to 1700 A.D. The same pattern of growth, he says, extends over a wide area. This is in contrast with the work of Antevs (8, 9) who recognizes a certain uniformity within a locality but who realizes the great variation in rainfall from one district to another and its undoubted effect on tree growth. A trend of tree growth taken among various groups used by Keen gives a parallelism of 29% between some, up through different percentages to 78% at the most. As a matter of interest, the rainfall of the year 1925 was picked at random from the Climatological Data of the United States for Eastern Oregon. Out of 51 stations, 26 had below normal rainfall, 19 had above, and 6 were about normal. For 1923, 49 stations: 13 below, 35 above, and 1 normal. For 1921, 47 stations: 7 below, 36 above, and 4 normal. For 1917, 42 stations: 29 below, 19 above, and 4 normal. The figures show that the "broad climatic zone" of Keen probably does exist in a generalized fashion for certain years but *not for others.* Such facts must give pause to the student who desires to read past rainfall from the widths of growth layers. And rainfall recorded is by no means soil moisture where and when the trees need it. Antevs, as mentioned, prefers to make tree growth-rainfall comparisons locally whereas Keen covered a large diverse region. Kolmodin (121) thinks that variations follow a certain law, as it were: the closer the collecting areas are to each other, the more the relative annual-ring curves approach each other.

Locally there has been a curious mixture of statement, on the

one hand cautioning (64) against application of the results from one region to another, and on the other hand making such application (52, 54, 171). Also, curiously enough, long distance cross-dating (48, 49, 50) was attempted by Mrs. De Geer between the trees and glacial varves of Sweden and the sequoias of California, whereas different calendars (185) had to be set up in New Mexico. Mrs. De Geer's cross-dating would seem premature until a great deal of fundamental work on weather areas, like that of Ångström (6), shall have been done. "In the pines of northern Arizona the same years may be readily identified across hundreds of miles of country . . ." (52), and yet, within the area mentioned as well as on its borders, not only do marked variations of growth-layer sequences occur, but also many, many sequences exist which do not fit into the one scheme. Keen (116) in eastern Oregon obtains no statistical correlation with the Big Trees of the Sierra Nevada but does get a correlation with certain Arizona pines. All of which, if justified, may be found to indicate that here and there on the earth's surface, just as here and there within a single region, growing conditions have a rough similarity during occasional years.

Cross-dating indicates either a like fluctuation in limiting factors or similar responses to compensating factors. The assumption that cross-dating proves the annual character of growth layers requires that those factors which control cambial activity fluctuate on an annual basis only. A consideration of the one factor, soil moisture, indicates the futility of assumption as to cambial activity without experiment (39, 40, 41, 137, 138, 156, 157, 158, 159, 162, 164, 175, 178, 179).

Cross-dating does exist, there is no doubt. It is essentially a local affair (85, 86); physiologically and ecologically the conclusion is inescapable. It exists in all ranges of quality; experience has amply shown such to be the case. Has it been misunderstood? Has its uniqueness completely overshadowed what the trees are actually recording? On the whole, the following statements by Chapman (32) are apt to hinder rather than aid understanding and research: ". . . the tree ring calendar . . . has undisputed validity. On no other hypothesis than the variation in annual rainfall can the coincidence of broad and narrow rings in these trees be explained." No one else apparently has ventured quite so far.

The assumptions that cross-dating is the basis for climatic [*i.e.*,

rainfall] interpretations and that cross-dating proves the annual identity of the growth layers, are, at present, in grave danger of complete refutation, at least in those regions where the matter has been investigated. Clearly the assumption relative to climate will not merit serious consideration until verified by the "ecologically minded botanist." The elucidation of the intergradations and complete divergence within a single region, or even area, depends upon basic research, not the type mentioned by Haury (100) whereby more master calenders should be set up, but the type whereby the physiological functions, the ecological influences, and the anatomical responses fundamental to tree growth shall be more thoroughly investigated.

STATISTICAL CORRELATION

The custom has become increasingly prevalent during recent years of using statistics in biology. To many of us working in biological problems the statistics become so intricate that we either disregard them or place unjustified reliance upon them. For population and kindred problems the Pearsonian methods, as modified by Fisher (68), have high value as suggestive of relationships or directive for further inquiry. A misunderstanding of the derivation and purpose of the statistics may lead not only to their illegitimate use but also to unjustified interpretations. Since the Pearsonian method applies to data on population samples, it is necessary to bring out how the method has recently been employed in tree growth-rainfall comparisons, a different type of data which constitutes a continuous time series.

The use of statistics or mere mention of the correlation coefficient, r, has come to be the vogue in the work of many (38, 46, 51, 55, 58, 66, 80, 84, 89, 97, 98, 102, 116, 124, 134, 173, 174). Douglass (54) and Keen (116) avoid placing too much reliance on statistical comparisons. Later on, however, Douglass (55, 58) seems to rely more and more, perhaps unwillingly, upon the mere mention of a coefficient. In contrast, others (9, 113, 125, 129, 131) do not resort to correlation, which could add nothing to the thorough analysis of Antevs or the careful and arduous work of Lathe and McCallum.

At first Lyon does not resort to statistics; in 1940 (134), he adds them to his own particular methods of comparison. He regards

correlation as acceptable proof of the *influence* of temperature and rainfall upon growth. The crux of his work seems to be his obvious and healthy reluctance to substitute statistics for an understanding of the physiology of growth. Goldthwait and Lyon (89) leave the reader somewhat in doubt as to what they consider a positive correlation, and one can not check the work because actual values of r, as well as ring measures, are not given. Diller (51) finds a disconcerting mixture of lag and no lag in his comparison of rainfall and tree growth. This uncertain lag effect "together with the fact that rainfall is an exceedingly variable factor throughout a given region, makes it practically impossible to correlate statistically the relation between rainfall and growth."

In the statements of Hawley (102), who relies strongly upon statistics, an interesting practice comes to light: "Correlations were computed for the 22-year period, 1911–1932, excluding the year 1920 in precipitation correlations, for which year the data are erratic." The period is rather short and one wonders about the omission of a year, especially in view of the remark by Erlandsson (66) on the work of Eide that the omission of the tree growth for 1915 raised the correlation coefficient from 0.62 to 0.81. Coile (38) obtains a value, $r = +0.750$, between tree growth at Waycross, Georgia, and February–April rainfall for the interval 1918–1933. "If the annual fluctuations of rainfall for the years 1921 to 1927, inclusive, are disregarded, almost complete agreement with growth obtains." But this leaves only 9 years out of the 16 with "almost complete agreement." Then, too, the years 1927–1929 compare rather poorly. Six years of good agreement out of 16 does not furnish encouragement to other workers who would employ such agreement as a basis for extrapolation.

A few biologists apparently are willing to give statistics a thorough trial. For example, Coile (38), although realizing the limitations of statistics, resorts to correlation only when graphic methods appear unsatisfactory. Erlandsson (66) shifts his graphs by successive years up to 6 years before, and 6 years after, the supposedly corresponding dates, and finds a real relation only with the July temperature of the year before. However, the odd years in the series give a relatively high correlation, indicating simply the rhythm of the well known 2-year cycle. It is of particular interest to note that Keen (116) obtains a correlation of $+0.41$, which he

considers significant, between his pines of eastern Oregon and those of Douglass in Arizona. The pertinent query immediately arises as to what is the "significance" of such a rough correspondence of growth variations in terms of the trees or in terms of growth factors. Without doubt, climatic factors, soil-moisture fluctuations or other growth conditions show a somewhat crude parallelism of variation for certain areas at certain intervals. To read further significance into such comparisons at this time is questionable practice and probably should be held in abeyance until the causes and consistency of parallelism shall have been rather thoroughly investigated.

Lyon (131) does not expect nearly perfect correlations because he recognizes rightly many interrelated factors at work on the trees. Fair correlations over a long period, he thinks, are better than good correlations over a short period. Finally, he says he can not employ the usual statistical methods because his graphs show a lag effect here and there. Schumacher and Day (173) and Schumacher and Meyer (174) make a thoroughly intelligent use of pure statistics although one will have difficulty in following the intricacies unless he has special mathematical training. They (174) give no graphs of tree growth and rainfall; hence, the results must be accepted or rejected on the basis of statistics. The statistical tests are excellent if the data and assumptions are well taken.

As a matter of fact, a few voices have been raised against the use of statistics for certain types of biological work. Ashby (11), from his standpoint, says frankly that "many applications of statistics to the analysis of vegetation must be regarded as signs of decadence rather than of progress." Such applies unfortunately where statistics are used as a substitute for strict observational and experimental analyses. With equal frankness Muller (149) says ". . . the relationship between climatic and biologic phenomena does not lend itself to solution by mathematical formulae." Sampson (168) is no less critical. "A few close relations between variations in width of tree rings and annual[1] rainfall appear to exist for certain areas, according to a particular interpretation technique. But even so, it is faulty logic of a high order to conclude that the same

[1] Koehler (120) objects to the fact that Davis and Sampson compare tree growth with *annual* rainfall because only that rainfall which affects the soil moisture of the growing season can have any effect on growth. In truth, Davis and Sampson (46) did try various rainfall periods and found annual rainfall to compare most favorably with tree growth.

relations exist for periods (56, 103, 169, 170) or areas (185) with no available climatic records. That such relationships 'look probable' affords no evidence as to how reliable they may be or actually are. In the absence of climatic and much other data, obviously no statistical treatment is possible."

Objections have been voiced in connection with the details of sampling. Large collections are made in the field only to be drastically reduced when the growth-layer sequences are examined in the laboratory. Hardman (97) selected 46 sequences out of 200 collected. Keen (116) collected 1240 specimens from 44 localities. Twelve to 15 cores were selected at random from each plot, and from these 10 of the more uniform cores were measured, thus totalling 440 samples. He believes 10 in a group just as good as a larger number. In contrast, Lathe and McCallum (125) used 100 or more in a group because they found 25 insufficient. Lassetter (111) used 25 out of a collection of 640, and from the curve of these 25 he developed equations for the derivation of precipitation and runoff. Antevs (9) used 19 trees out of the 100 which he collected, but he himself did not make the laboratory selection. No mention is made why 81 were rejected. A question arises, therefore, because Antevs undoubtedly collected the samples with his usual thoroughness and his characteristic comprehension of all factors involved.

Lyon (134) discarded all trees which did not agree with their groups. Such trees were held to be poor indicators of site conditions, an assumption of grave concern to scientists. Until growth factors and response (24) shall have been completely investigated, cross-dating can not be considered a "magical resource" which places non-cooperating trees, so to speak, outside the bounds of legitimate ecologic response. In contrast to the foregoing, Lathe and McCallum (125) practiced no selection except in the field; they tried to eliminate the personal factor. Their methods of collection and the number of samples, over 10,000, complied well with statistical principles, and yet, significantly enough, they do not resort to statistics.

Sampson (168) says forthright that the selection of trees for cross-dating initiates statistical unsoundness, and, as before, botanists wonder with Sampson why only a certain few trees should have climatic value. He notes close correspondence between tree growth and rainfall only in rare instances. "This lack of a sound starting

point raises the question as to how accurately tree rings reflect rainfall at the present time. Obviously, the inquiry must be left unanswered, due to lack of statistically sound data, since the accuracy that has often been claimed for correlations of tree-ring and precipitation data shows a misuse of statistical methods." From the standpoint of statistics and from the standpoint of cross-dating in regard to ecologic response, it is significant to note that Haury (100) was able to "date" only 18 out of 60 rooms in an Indian ruin. The rest of the rooms did not contain datable material.

"Tree-ring" work, as it has been commonly carried on, most certainly has not followed the statistical principle of random sampling (167). However, there is another feature, in addition to the lack of representative and random sampling, which militates against the use of statistics, and that is the short length of record. Sometimes as few as 7 to 17 years are employed. Even 50 to 60 years give no legitimate basis for deriving rainfall over a 2000-year interval.

It is to be remembered that the statistics were invented for a very definite object, the sampling of populations. Tree growth-rainfall correlations, by contrast, involve a continuous time series. J. Bartels of Germany stated in conversation that Pearsonian methods of correlation could not be applied legitimately to continuous time series. Croxton and Cowden (44) call attention first to the few extreme deviations in time series which largely determine the value of r, and second to the lack of any logical basis for estimating the reliability of the coefficient of correlation because the observations are not randomly distributed. They say, further: "The coefficient of correlation must be thought of, not as something that proves causation, but only as a measure of co-variation."

Snedecor (181) reviews the principles of statistical sampling and then says: "Statistics are no substitute for common sense and a statistical treatment of poorly drawn nonrepresentative samples is not only a waste of time but may be seriously misleading. . . . The mere existence of correlation is no proof of direct relationship. . . . It is dangerous to deduce relationships from correlations. On the other hand, it is good practice to use correlations for testing or evaluating suspected relationships." The principles of representative sampling must be followed. Therefore, it would seem that statistical methods can not be applied legitimately to the tree growth-

rainfall comparisons, and, if they could, they would merely suggest a relationship and not a causal connection. All to be said for so much of the work which has been done is that it suggests the strong influence of water supply upon tree growth, something known to botanists for many years. The existence of so-called statistically significant correlation, then, gives no justification whatsoever for the derivation of past or future rainfall. Sampson's stand (167, 168) must be considered as well founded, and, in the long run, reliance will be placed on basic botanical research, for there is no magical "short-cut" (44) to knowledge of the past or future.

If more rainfall means more growth, then surely a parallelism in the direction of the response is to be expected (137), and a simple enumeration of the years during which the graphs of tree growth and of rainfall go up and down together gives a fair idea of corresponding variation. Compare this with the statistical method where a mean line is used. Tree growth can decrease by a large amount and rainfall can increase by a large amount; if both points come to be on the same side of the mean line, their product, in spite of the opposed direction of response, will enter the calculations as a positive quantity. The existence of just such responses in the derivation of r led Glock to doubt the validity of statistical correlation and to work out (79) what he called the trend coefficient, t, for use especially with time series.

In the trend method no mean line is used. Departures are obtained by computing the difference in position of each two successive points on a graph above the baseline. If the second year is an increase from the first, the departure is plus; if a decrease, minus. These departures for tree growth and rainfall, as x and y respectively, are multiplied. The ratio of the algebraic sum of xy's to the total sum of xy's, regardless of sign (to get total sum), gives the coefficient, t.

$$\frac{\text{Algebraic } \Sigma xy}{\text{Total } \Sigma xy} = t$$

If $t = 0.00$, parallel and opposite trends are equal; if $t = 1.00$, the variations of the two graphs are parallel throughout; and if $t = -1.00$, the variations are completely opposed. The trend coefficient, it is thought, is comparatively independent of mean line and secular trend.

Here again, as in the case of r, a few departures of large ampli-

tude could outweigh many of small amplitude. An index, i, to reveal the amplitude and the number of cases has been devised. Let $n(+xy)$ = the number of cases of parallel trend, and $n(-xy)$ = the number of cases of opposite trend. Then the ratio of the average amplitude of parallel departures to opposite departures gives the index, i.

$$\frac{\dfrac{\Sigma + xy}{n(+xy)}}{\dfrac{\Sigma - xy}{n(-xy)}} = \frac{\Sigma + xy \cdot n(-xy)}{\Sigma - xy \cdot n(+xy)} = i$$

If i is greater than 1.00, the amplitudes of the parallel trends are greater than the amplitudes of the opposite. The index has its highest value, however, where the ratio of $n(+xy)$ to $n(-xy)$ is also stated. Trend relationships may be shown graphically by plotting x and y values on coordinates, thus giving the scatter diagram. The trend coefficient, it is thought, is a legitimate method of comparison for continuous time series. It indicates the degree of parallel variation, but it does not indicate causation. Even if the mathematical advantages of the trend coefficient should prove to be slight, the fact remains that the computation of t consumes far less time than the computation of r.

SMOOTHING

A description of the many mathematical manipulations to which growth-layer measurements have been subjected has little place in discussion at present. Because of the rather vital rôle smoothing may play in comparisons of tree growth and rainfall, it is touched upon briefly.

Most students appear to prefer the use of original [or, raw] rather than smoothed data, and the practice has a great deal in its favor. Others use some form of smoothing without explanation (9, 55, 58, 82, 196). Hardman (97) smooths his data because of lag effects and because he wishes to decrease the amplitude of the highs and lows. Hardman and Reil (98) say: "This treatment takes out the extreme variations and renders the trends in the curves more readily comparable." Girand (78) favors the Hahn method whereby the middle member is given double weight in contrast with a running mean where single weight is allowed. However, he says the resultant should be on the last term of the three

Hawley (102) prefers the Hahn method. "When the Hahn formula is applied to . . . tree growth, run-off, and precipitation curves, it is found that higher correlations appear between Hahned curves than between curves smoothed" by any of several designated methods. Should this be the standard of selection? If graphs are smoothed, Jessup (113) states, they show better agreement. Such result is inevitable because of the nature of smoothing. Keen (116) smooths his graphs on a 5-year average in order to get general comparisons above and below the mean. However, his graphs on percentage coordinates do not look balanced with respect to the mean line.

A few students resort to successive smoothing. Antevs (9) smoothed all graphs of tree growth twice. Hardman (97) and Hardman and Reil (98) take running, or moving averages of 5 and 3, and place the resultant on the mid year. Douglass (55), in reporting the work of Glock, mentions a correlation coefficient of +0.50 to +0.55 between tree growth and rainfall unsmoothed. When smoothing and a lag increment are applied, r increases to +0.70 or +0.75. Further cumulative smoothing gives r as +0.80 to +0.91. By successive smoothing, a graph approaches closer and closer to a straight line. Quite naturally, then, the correlation coefficient between two graphs will increase as they are smoothed once or several times.

CYCLES

In very recent years there has been, in general, a decreasing emphasis on cycles although their importance is of the highest order. A few papers are devoted nearly or quite exclusively to a consideration of cycles (2, 17, 17*a*, 35, 58, 140, 187). Most students do not mention, whereas others on the whole refer to them rather briefly (14, 46, 47, 49, 50, 66, 71, 82, 97, 104, 116, 120, 121, 131, 144, 161, 165). This no doubt is due to several circumstances:

1) Little success has attended the efforts of meteorologists to resolve the variations in rainfall into understandable cycles. Bowman (18), quoting Kincer, says: "There is no 'apparent conformity to any law of succession' in rainfall variation." C. D. Reed is reported (161) to have found precipitation cycles in the records for individual stations but to have had no success in the averages for large areas. The work of C. G. Abbot in the last ten years supports

Reed's results for individual stations. If rainfall cycles are expected to appear in tree growth, it is logical to hunt for them first in the rainfall records. A few workers have gone forward on the assumption that cycles in tree growth do give an accurate picture of cycles in rainfall. Sampson (165) summarizes the matter in this way: "unless cycles are shown to be reasonably dependable for periods for which climatic records are available, they can have little other than speculative value as past and future climatic indicators." Therefore, the results of meteorological research must always form the point of departure for those seeking to work with cycles in growth-layer measurements.

2) Many (17, 116, 131) find cycles or cycle effects not in evidence or of little significance. From an analysis of cycle length, Keen concludes that "the range of these intervals is from 19 to 219 years, so the regularity of occurrence is certainly not significant."

3) Some work (58) in tree growth has yielded almost a hopeless number of so-called cycles, discontinuous, variable in wave length, and simultaneous and superimposed or overlapping. In some cases, more than one cycle exists for each whole year number, or fraction thereof, along the range examined. Several weak cycles, in phase for a short interval, might be mistaken for a strong but discontinuous cycle.

4) Most workers acknowledge the influence of many factors upon tree growth, even though they at once confine themselves to climate. This in turn seems for most of us to mean rainfall. Stetson (186) seems just a bit sceptical on the subject of sunspot cycles and especially rainfall cycles in tree growth. He mentions "that temperature, the quality of sunlight, and particularly the percentage of ultraviolet enter into the growth rate of trees as well as does rainfall." Unless a person disregard present knowledge in plant physiology, anatomy and ecology, and declare with Hawley and Clark (103) that in tree rings with their patterns of variable widths we have "the perfect, the indisputable, record of climate . . . ," he recognizes the complexity involved in xylem formation. How may we determine what part of a graph of growth-layer measurements is controlled by climate? What part of the climatic portion is controlled by rainfall? To which period within the rainfall year does the rainfall portion apply? If the fluctuations in a graph should be controlled, say 60% (*i.e.*, $r = +0.60$), by rainfall, how would this

amount of control be revealed in the cycles? A cycle controlled 60% by rainfall—does such a condition have any reality?

5) A few attempts have been made to link sunspot cycles either directly to tree growth or indirectly through the medium of the weather. Little success has resulted, chiefly because the relation of weather (186) to sunspots is highly complex and because the sunspot cycle has varied from 7 to 17 years in the last 2 centuries. If a relation exists between the sunspot cycle and tree growth, the trees must tend to follow the cycle whether it is 7 or 17 years. Therefore, unless trees are able completely to disregard the variations in the length of the sunspot cycle and can average those cycles over long intervals at values of 11.2–11.4 years, we can hardly be justified in calling a consistent 11 + year cycle in the past a sunspot cycle. The problem as to the existence of a fundamental solar cycle of 11 + years, apart from the so-called sunspot cycle, depends upon astronomic research.

6) Finally, a great deal more fundamental knowledge on tree growth in general and growth response in particular is necessary before cycle studies can have their full significance. The water falling as rain, for instance, is a long way from that used as cell sap (46, 147, 168). Davis and Sampson (46) remind us that physiological activity of a tree cannot be plotted as a function of the rainfall on the soil surface. Nutritional functions are highly complex and the deposition of cellulose depends upon many chemical reactions (168). Variations in the deposition of cellulose may show the influence of variations in soil moisture, but great hazard attends the assumption that they depict variations of soil moisture in any except a modified form.

Glock (82) attempts to bring out a general utility in cyclic fluctuations by saying that short, high amplitude fluctuations characterize dry climates, and long, low amplitude fluctuations characterize wet climates. He bases his statements on the observation that trees exist where they have either minimal, maximal or optimal amounts of water supply (85). Antevs (9, 10) and Bowman (18, 19), in their use of growth layers to reveal general conditions favorable or unfavorable to growth, have, it seems, carried their interpretations to a point consistent with present botanical knowledge and scientific ideals.

On the whole, work on cycles in tree growth must proceed on

assumptions as long as it is in advance of meteorological and astronomical research and botanical investigations into growth response.

SAMPLES AS REPRESENTATIVE OF GROWTH INCREMENT

Because cambial activity and xylem formation are by no means uniform over an entire tree (3, 69, 70, 82, 88, 93, 137, 158, 177), some attention must be given to the type of samples from which attempts are made to obtain so much of rainfall and climatic history. The geometrical figure of a tree possesses rather intricate combinations (111). It is not a simple cylinder whose volume is wholly a function of one radius. Anything less than the whole tree produces a quantitative distortion of varying degree.

So far as can be determined, a few students base their comparisons of tree growth with precipitation or runoff on a single increment core, of about 5 mm. diameter, from each tree (38, 77, 97, 98, 102, 116, 121, 125). Coile (38) thinks one core per tree is sufficient because the work of Lodewick shows one core to be about as reliable as an average of four. The cores Coile used were collected by someone else and measured wet in the field; thus, "possible errors due to shrinkage or contortion of the cores as a result of drying were eliminated." In this connection MacDougal (137) noted much shrinkage when the outer layers of the xylem were exposed. When measured, cores should be either saturated or dry, and not at some degree of wetness between the two extremes. Lathe and McCallum (125) took single cores, it is true, but the total number taken, over 10,000, overshadows to a great extent whatever of linear distortion a few cores could bring about.

Sperry (183) took two cores, one from the north side and one from the south. South slope trees (182) he found had greater growth on the north side. Diller (51) took three cores out of each tree systematically from the directions north, southeast and southwest. Such practice of using certain compass directions appears somewhat arbitrary and unnatural if rigidly followed.

Some workers give no information on how or where cores are taken. At best, a single core, or even several of them, is merely an exploratory sample. How futile and misleading it may be to make interpretations on the basis of cores becomes quite obvious when one reads "cores collected in early May showed that no diametral increase for 1939 had yet begun" (171). To be sure, no

cambial division may have taken place on the tiny cross-sectional area of the one core. Botanists know well that cambial division does not necessarily occur on all parts of branches, bole and roots at the same time. Furthermore, to detect the early stages of cambial division requires refined botanical technique and magnifications much higher than those of a hand lens.

Radial, or V-, cuts reveal a better picture of a trunk than a core in proportion to the greater volume of wood included. Antevs (9) and Davis and Sampson (46) used single radials. Others (102, 116) mixed radials with the cores.

Transverse sections yield a fair percentage of the trunk volume although their weight and portability are handicaps. Of course, the entire sections are not used. Much depends on the selection of the particular radii to be measured, and it is difficult to see upon what basis, aside from injury, one radius rather than any other is supposed to represent the record of the tree's response to its environment. The average of three selected radii (72, 89, 129, 131, 134, 174) constitutes a better representation of growth, it is thought, than an equal number of cores. Jessup (113) averaged 4 radii. Schumacher and Day (173) measured from 2 to 4 radii. Kleine, Potzger and Friesner (119) measured 8 equidistant radii on each section. Lyon (129) chose his 3 radii so as to avoid flare. He objects to increment cores and insists on using mature rings only. Under what has been considered (58) the best of conditions, Glock (82), working on transverse sections, recommends the use of 6 radii, certainly no less than 4, in gaining a fairly accurate linear measure of diameter growth.

Mrs. De Geer (50) deliberately selected one radius. She chose "sections of well-developed rings only, where growth has proceeded unchecked by accidental hindrances. Such rings furnish a true representation of the climatic reactions of the tree. Radii in which growth has been retarded are misleading on account of the closeness of the rings." On what evidence can we consider rings a true representation of climatic reaction? Erlandsson (66) measured one radius, that to the south. "In many trunks the annual rings are distinctly developed only on the south side of the tree, and on the north side they almost run together or are not to be found at all." Weakley (197) found 1 out of 4 juniper sections to have sufficient symmetry to be usable. On the whole, the use of sections

appears to have little advantage over the use of one or more cores, unless several radii are averaged. Deliberate selection of radius replaces chance by boring and assumes that the collector has complete knowledge of the type of response a particular tree should give to its environment. And commonly the selection occurs in the laboratory.

Linear measurements of growth layers also encounter the difficulty of variable widths (177) in the different parts of a tree. MacDougal (137) found much variation around the circuit of a single tree, as revealed by 6 cores, each from a different direction, and Haberlandt (94) reports much work by others on longitudinal differences. Working in the coastal region of California, MacDougal (137) and Shreve (177) noted marked variability in the width of growth layers throughout the trunk. Jaccard (111) noted the same in Switzerland. In contrast, Douglass (58) and Glock (82) in Arizona found sufficient agreement at different trunk heights to permit the matching of growth layers. The difference between a rough uniformity in some regions and variability in others depends either upon site factors or upon species, with experience indicating the former as the more likely. Field work points strongly to the conclusion that variability in absolute thickness as well as a reversal in relative thickness between two contiguous layers in a tree characterize regions of maximal (85) or low minimal amounts of soil moisture.

Question has arisen as to how accurately one radius, or several, shows volume increase. Sperry (183) recognizes that "if the diameter increase for any year is as great or greater than for the preceding year, the total increment for the later year is greater." Adams (3) states: "Although height and diameter growth are component measurements of the volume growth, the more accurate index of the trees' development is shown by the yearly or periodic volume increment." For instance, MacDougal (137) noted the addition of wood to the upper part of the trunk and to the branches of pine and redwood after radial growth had ceased at the base. He (138) also calls attention to the roots, proposing "to include the volume of the roots and branches in estimates of tree growth, and to characterize the season by the thickness of the layer which the leaf products would form if applied to the surfaces of the leaves carried." Avery, Creighton and Hock [Amer. Jour. Bot. **27**: 825–

831. 1940] use linear measurements but at the same time recognize that volume increment is a different problem. They compute areas of growth layers on a section and say: "The areas, and therefore presumably the volume of the rings, continued to increase as the trees increased in age" in spite of decreasing thickness of growth layers. Even area does not reveal true volume changes. Certainly, total volume is the only true measure of tree growth (177).

It is quite clear, then, that one or more cores or one or more radii taken from the trunk yield a rather rough approximation only of the total xylem increment set down during a growing season or distinct portion thereof. In many cases the approximation may be highly inaccurate. Cambial activity over the entire organism is far too complex to have the results measured in linear fashion along one radius of the trunk, especially when the measurements are taken to hundredths of a millimeter. The methods of sampling individual trees leave something to be desired.

FACTORS AT A PARTICULAR SITE

Attempts to find positive relationships between tree growth and rainfall in the belief that they can be used as a basis for the derivation of past and future rainfall have become so common that the protests voiced against the methods employed have gone unheeded in many cases. Ecologic principles seem to have been disregarded, first, because of the high selectivity exercised not only in the field but later again in the laboratory, and second, because of the belief that only certain trees of an apparently homogeneous group respond to their climatic environment. It is necessary to record that thin and rocky soils, steep slopes, ridge tops, and forest border areas, where "violent changes from year to year" occur in the rainfall (170), are considered the proper sites to support trees which yield a readable, dependable chronicle of climate or rainfall. These field and laboratory practices strike at things fundamental; they have engendered a measure of doubt in the minds of botanists, foresters and horticulturists.

The urge admittedly has been to secure tree growth-rainfall correlations. For such purpose, only trees showing a rough correspondence of growth fluctuations are permitted to contribute to the record because they alone, it is said, respond to rainfall. The fac-

tors of a particular site may be dominated by a single one but it is the whole complex that constitutes the microclimate, or better, the microhabitat, of a single tree. Each tree, in a fashion, responds to the algebraic sum of all contributing factors of its microhabitats. "Since the growth of a tree depends upon its reaction to its environmental conditions, it is obvious that there exist as many sites as there are combinations of the single factors which affect it" (24). In a stand, certain algebraic sums have a rough similarity, thus causing a rough correspondence in tree growth which is commonly ascribed by some workers to the rainfall. Others have realized not only the primary importance of water to plant life but also the complexities of contributing and compensating factors when attempts are made to isolate the effects of a single factor except by controlled experiment. Those students (3, 9, 23, 24, 33, 43, 46, 66, 71, 89, 137, 138, 152, 167, 173, 174, 196) who have paused to inquire into the nature of growth response have not reached the stage where single factors can be correlated. When we come to understand growth response, correlation may be unnecessary. Apparently (185), botanists have been "frustrated" by the complexity of growth factors, whereas others have not been.

Kuechler in 1859 (117) took his oak trees from high isolated positions. In the same manner, Douglass (58, 60, 63) some 70 years later preferred trees from the forest border where soils are thin and rocky and where rainfall for the species is at a minimum. In regard to Monterey pine he says (60) they were selected so "deficiency of precipitation should be promptly felt by the trees; thus thin soil, steep slopes, and some isolation from neighboring trees emphasized the deficiency." Perhaps they exaggerated rather than emphasized the deficiency. Hardman (97) took trees from well-drained sites on the lower margin of the forest. In the area sampled by Hardman and Reil (98) along the eastern foothills of the Sierra Nevada, the drainage is good, the soils very pervious and shallow. The trees came from hill slopes or ridge tops. Antevs (9) worked at the edge of the Great Basin. Some of his trees grew on very steep, dry slopes covered with cobbles and boulders of lava; others grew on gentle slopes of rather dry ridges. Davis and Sampson (46) used trees which grew on steep slopes with uniform soil and exposure.

Kleine, Potzger and Friesner (119), working in Indiana, state:

"All [sections] were taken from the Knobs area in which run-off is always high and soil moisture is always near the critical point during summer months." In New England in 1936, Lyon (131) chose trees from a mixture of sites; in 1940 (134) he chose those rooted in shallow soils over and beside a ledge with irregular outcrops. "When the measurements for any one tree were at variance in this matter of 'cross-identification' with other trees in a group, the tree was considered to be a poor indicator of growing conditions at the site." Might we not obtain valuable information from such "poor indicators" if we but knew how to treat them? Goldthwait and Lyon (89), choosing trees from typical sites, probably obtain a fair picture of growing conditions for the locality.

In contrast with much of the above, Lassetter (124) did not use cedars [sic] because they grew on cliffs and places with little soil. The observations of Brady (20) suggest basic facts about growth layers and the cambial activity which produces them. The trees he observed were rooted in small soil pockets in boulders which permitted none of the roots to reach into the general soil. Within a few days after a rain the soil was dust dry and presumably growth ceased. Site conditions here attain the extreme, and perhaps the trees give a good picture of the *nature* of the rainfall during their growing season. Only experimental methods strictly controlled can give us the true relationship between soil moisture and cambial activity.

Brady's work is a most suggestive bit of observation. Trees from border areas, from thin soils and rocky ridge tops show a supreme struggle in their own habitats where the interplay of growth factors incite, inhibit or barely permit cambial activity, sometimes continuously, other times intermittently. Surely, tree growth in habitats which exaggerate runoff, evaporation and soil-moisture depletion, can not be expected to give a clear picture of rainfall that strikes the surface of the ground, even if trees could record the water factor alone. Thus, extreme habitats give neither a representative picture of tree growth for a region nor a measure of the true rainfall.

GROWTH LAYERS AND ANNUAL INCREMENTS

Invaluable work on the understanding of trees by MacDougal (137, 138, 139) and by Priestley (158), on the relating of trees to

their environment by Pearson (152) and by Veihmeyer and Hendrickson (195), and on the summarizing of physiological knowledge, as by Meyer and Anderson (147), gives us indicative information about the relation of temperature and moisture to cambial activity. Oddly enough, the flurry of the past several years dwells upon comparisons of tree growth directly with rainfall. Records of rainfall are, to be sure, conveniently available. However, trees root in soil, and soil water as a solution supplies the tree with water and mineral food. All the factors of topography, soil, penetration of water, absorption by the roots, as well as those of air temperature and humidity, crown and root development, and nature of precipitation, stand between the actual rainfall and the water which takes its way along the roots toward the stem. And the water moving along the roots is still separated by many factors and processes from actual construction into cellulose. These facts can not be disregarded by those who seek a direct record of rainfall or of rainfall cycles in tree growth.

Adams (3) does not consider rainfall a true index of soil moisture, and Bowman (18) says the rise and fall of ground water only approximately follows increase or decrease in rainfall. Shreve (178) found only 1 rainy season out of 6 near Tucson, Arizona, which had influenced soil moisture below 60 cm. The effect of this "profound wetting was paradoxically to reduce rather than to increase moisture of the lower levels." A wet soil above, rather than dry, forms a better capillary system. Weaver (198) did not find soil moisture uniformly distributed, a fact to be expected from the nature of soil (164), the distribution of rains (39, 179), and unequal losses of water from the soil. Studies like those mentioned are of vital concern to tree growth, and serve to eliminate disappointment with tree growth-rainfall comparisons. But not the least instructive is the work of Veihmeyer and Hendrickson (195). Their experiments show the shallow penetration of water applied to the surface, as was observed by Shreve (178), and the extremely slow migration of soil water. "Such movement as occurs has been found in our tests to be extremely slow in rate and slight in both amount and extent."

The development of root systems is intimately associated with the occurrence of soil moisture. The upper 18 inches of a forest soil hold most of the available water, and here live the great mass

of roots (38). Yocum (203) gives us valuable suggestions toward the variations of cambial activity in different trees by his statement: "A tree of given age which most thoroughly occupies the largest volume of soil will be the one best fitted to withstand adverse conditions." Even so, roots must lengthen with sufficient speed to remain in contact with water (160) and maintain the transpirational stream, since soil moisture below field capacity can not migrate appreciably (164). If rainfall is not sufficient to replenish soil moisture, it may eventually become exhausted (21). Local variations in soil properties, variable infiltration capacity, and root extension must be considered at least as important in wood formation as the frequency and the amount of moisture replenishment (164). Hence, variations in root systems together with those in soil moisture may affect xylem formation in two adjacent trees which, therefore, will not cross-date. Both trees reflect their environment, and neither may justifiably be rejected on the basis of non-correlation.

Fluctuations of soil moisture have not been a neglected topic although a few writers do speculate about the condition of the soil moisture without investigation. In his work in New England, Adams (3) finds the soil to be nearly saturated at the start of the growing season. His soil-moisture graph for the 1931 season has a double minimum whereas the one for 1932 has several highs and lows. It is of course conceivable that available moisture can fall low enough in mid season to reduce cell turgor and interfere with cambial activity. Only long-continued investigations will show how often this occurs in New England. In contrast with New England, the soil moisture in California (93, 137, 138) seldom is replenished during the summer, and cambial activity often ceases, only to be resumed if autumn rains occur. MacDougal (137) shows us by his experiments on Monterey pine that irrigation in July brings on a second period of cambial activity after the first has ceased some time earlier because of soil-moisture depletion. The significance of MacDougal's experiments is clear in regard to the fluctuations of soil moisture and cambial activity within a season under certain conditions.

Graphs of soil moisture for the growing season in southwestern Texas by Cottle (40, 41) show a double maximum. Yocum (203), working in Nebraska, found two maxima in soil moisture, the first

one being the more intense. In eastern Oregon, Keen (116) thinks a total lack of soil moisture or complete saturation rarely, if ever, occurs for more than short periods of time. Pearson (152) speaks of the exhaustion of soil moisture early in July in northern Arizona. His graphs characteristically contain two maxima and reflect the influence of seasonal rainfall. Further, he states that "records in the western yellow pine have shown that for depths of 12 inches or less, complete or nearly complete exhaustion of growth water may usually be expected in the latter part of June." Closer to the forest border "far more extended depletion is the rule." These facts, coupled with the strong development of the surface root system and the shallow penetration of rain after the June drought, must be applied sooner or later to cambial activity or inactivity, as was done by MacDougal (137, 138).

Other factors in addition to variation of soil moisture have noticeable influence. If a plant uses water rapidly, more will be left behind at wilting than if it is used slowly (162). Serrano (175) found that greater amounts of moisture remain in clay loam in proportion to the greater age of rice plants. Old plants wilt earlier than young. He also found that the higher the air temperature is, the smaller the percentage of moisture the plants can extract from the soil before wilting. Soil moisture at or below the wilting percentage may even cause roots to exude water under certain conditions, according to Magistrad and Breazeale (142). Clearly, then, soil moisture itself is a highly complex factor in relation to the plant.

Of direct significance to students of growth layers are the results of Ishibe, as reported by MacDougal (138*b*). The starch reserves of *Pinus densiflora* in Japan fluctuated so as to give two maxima and two minima in the trunk and branches, and one maximum and one minimum in the roots. Wood formation depletes starch reserves (138*a*), and a check to cambial activity favors accumulation of starches (147).

Cambial activity most certainly can not be considered a simple process which begins as a unit over an entire tree on a definite date in the spring, runs a steady course for the growing season, and ends everywhere simultaneously in the autumn (137, 158, 199). According to MacDougal (137), cambium has no innate rhythm and no necessary period of rest. Periods of inactivity, however, may occur at any time during the "growing season" (3, 8, 13, 20, 81, 88, 114,

137, 138, 147, 163), something normally to be expected during certain seasons in different regions if soil moisture has anything to do with the hydrostatic system of plants (3, 20, 114, 115, 116, 119, 137, 138, 177, 203). Interestingly enough in this connection, MacDougal's (137) and Shreve's (177) observations on autumnal growth layers, as well as MacDougal's experiments with irrigation, suggest clearly the relation of soil-moisture variations to periods of cambial activity as one factor tending to produce multiple growth layers in a year. Antevs (8), remarking on a photograph of old wood published by Glock (82), calls attention to a thin growth layer which very probably represents a period of cambial activity in the autumn. If such autumnal growth layers exist, and no one has proved they can not, then, as Antevs points out in part, precise dating is impossible, wet and dry "years" in the past can not be identified, and supposed cycles are thrown out of step. Note should be taken: if growth layers record climate as accurately as a few suppose, then the autumnal growth layer of MacDougal and of Antevs will be rather generally present over an area. If the growth layer, considered annual by some, does not appear in a few specimens, it is assumed to be absent (82, 185), and preceding growth layers on those specimens are pushed back in date by one year. In fact, it is far more than a possibility that the autumnal layer of which Antevs speaks is a product of soil-moisture depletion and later replenishment within a single season, as described previously from the work of Pearson (152).

Recent work in west Texas by Glock and Reed (88) and by Glock (86, 87) goes even further in the matter of more than one growth layer per year. During the last two years Glock and Studhalter, working also on the southern High Plains, have been assembling evidence, which amounts practically to proof, for multiple growth layers in one annual increment. Characteristically, certain species grow more than one definite layer per year. The southern High Plains represent, as it were, an extreme forest border area. Whether or not trees will grow there indefinitely (21) may be doubtful. However, they have flourished for a number of years during which, at Lubbock, Texas, late spring frosts appear to have administered a worse blow to the trees than drought. The presence of multiple growth layers (8, 20, 88, 137, 138, 177) in one annual increment should be taken into consideration by those who take their trees from border areas (51, 57, 58, 61, 62, 63, 64, 185).

Brady (20) illuminated the forest border situation in a very clever manner when he studied ponderosa pine growing in small gravel pockets on boulders. "Examination showed that within a few days after a shower the soil in these cavities was 'dust dry,' so that one might expect a careful examination of the rings to show that many of them are multiple, representing growth during several wet periods within a single year and cessation of growth during the intervening droughts." Any region with summer rains, it seems, may have spurts of cambial activity and periods of rest, even in New England if one is to judge on soil-moisture studies (3). The works of many (3, 7, 8, 13, 86–88, 136–139, 151–153, 162, 164, 177, 178, 195, 203) indicate plainly that a study of soil moisture is one of the means to an understanding of the time or period wherein growth layers are formed.

Because of suppression, defoliation, low temperature, insufficient nourishment, soil-moisture depletion and other reasons, cambial inactivity can be prolonged throughout much of a season or even longer. Cambial inactivity, in fact, may extend over several seasons in certain parts of the trunk (5, 5*b*, 7, 67, 137, 138). The prevalence of "missing" growth layers in a forest stand depends, of course, upon the causative factor. If that factor is soil-moisture fluctuation, then *a growth layer, constituting but part of an annual increment, can be absent from a portion of the trees of a stand as it can be absent over the portion of a single tree from which a sample is taken.* Cross-dating may reveal the absence, and it can do so whether the growth layer is part of, or a complete, annual increment.

"Dendrochronologists" assume that every sharply bounded growth layer, as seen commonly under a hand lens on core or section, constitutes an annual increment (52, 56, 58, 64, 82, 100, 103, 185), and that every non-annual reveals itself by a certain haziness (62, 63, 64, 82, 83). The exact dating of growth layers to the very year (52, 56, 59, 100, 101, 170, 185) assumes of necessity that cambial activity can not come to rest completely within the general growing season, and if it does, it can not resume activity until the next year. It further assumes that the cessation of cambial activity at the end of the entire season causes a sharp outer surface on the dense wood. The work of MacDougal (139) and current work of Studhalter and Glock suggest that a sharp outer surface may not always characterize the annual increment.

Cross-dating, in its turn, assumes that growth factors influencing the amount of cambial activity in a season can fluctuate on an annual basis only. It is conceivable, of course, that these and other assumptions may ultimately prove to be correct. However, present knowledge of cambial activity and of soil-moisture fluctuations, to mention but two allied departments in botanical research, suggests strongly that the present belief of "dendrochronologists" in regard to accuracy of dating and significance of cross-dating requires modification. If so, much work dependent upon supposed annual dates and annual widths of growth layers will need revision. The factor of inaccuracy for each region can be estimated only after extensive investigation. Some will have a high factor, others low or none at all. Certain types of climate, such as in the central and southern Sierra Nevada of California (71, 85, 86), may seldom if ever produce more than one growth layer a year except perhaps in cases of partial defoliation. It is to be noted in passing that every one attempting tree growth-rainfall comparisons has had to assume the responsibility of identifying the annual increment. Schulman (171), however, reverses the usual procedure of comparisons from tree growth to rainfall and employs what he judges to be a favorable comparison of winter rainfall with growth-layer widths to prove the *annual* character of the growth layers.

Everyone wishes that the following statement of Hawley and Clark (103) were true: "The trees write their own clear records of the years and of late men have learned to read them far into the past." The first half of the quotation may have some truth on its side but the second half seems to be merely a hope unfulfilled. Interpretations are premature until the processes of cambial activity and growth response are better understood. Furthermore, rather than use unmodified rainfall data in connection with growth studies, we may do well to follow the implied suggestion in the words of Pearson (152): "soil moisture data are still to be rated as our best means of studying moisture conditions in relation to the growth of plants," and from there work onward. If soil moisture fluctuations have any bearing upon cambial activity, then multiple growth layers in one year may not be so rare after all. Multiple layers are no doubt less common in the basal trunk than in the branches, but if so, the trunk fails to give an adequate record of soil-moisture fluctuations. An understanding of tree growth depends, in part, upon

studies of cambial activity, soil moisture, starch reserves, growth activators, and their fluctuations. After having made such studies we may be better able to evaluate mutually dependent, mutually interacting factors.

TEMPERATURE CORRESPONDENCE

Relatively few students consider the temperature relation; at least they do not mention it. Others dismiss it either because little or no relationship is evident or because temperature variations are too small (46, 49, 78, 89, 116, 131, 137, 177). Those who attempt correlations find them unsatisfactory, diverse and sometimes contradictory. No doubt this is as it should be, first, because temperature effects in biochemical processes are not simple, even should we momentarily disregard the interaction of other factors, and second, because the influence of temperature may be direct as in initiation of cambial activity or indirect as in evaporation, transpiration and humidity.

Kolmodin (121) finds a rather good direct agreement of volume increment with summer temperature in the northern part of the Dalecarlia region of south-central Sweden. However, in central Dalecarlia and on to the south high summer temperature seems to be harmful to diameter growth. High rainfall with either high or low temperature gives large growth. Certain years are favorably warm whereas others are adversely warm. Erlandsson (66) worked in northern Sweden and Finland where he finds a direct influence of July temperature on tree growth. Correlation ranged from +0.46 to +0.77. A trend analysis gives 35 parallel trends and 17 opposite. Erlandsson himself cites exceptions to the general agreement. He finds a 23-year cycle in July temperatures but when he tries to derive the summer temperatures of the past, growth-layer widths do not lend themselves well to temperature calculations.

In eastern Canada, Hale and Prince (95) find the trend in the relation of basic wood increment to thermal efficiency in white spruce opposite to that for black spruce and balsam fir. In New England, Goldthwait and Lyon (89) cite evidence for a direct relation between tree growth and mean daily temperature for April–May, and for a possible inverse relation with June temperature. They do not consider temperature effects as important as those of water supply. Lyon (131, 133, 134) looks in vain for consistent

relationships between tree growth and temperature of the growing season. He finds, however, a positive relation between March–April temperature and tree growth. Why this should be so, he does not know, unless it be through an indirect influence on water supply in frozen as against unfrozen soil. Lyon realizes that a knowledge of the start and cessation of cambial activity is of vital concern to an understanding of temperature effects. He might well have included any one of the other growth factors.

In northern Indiana, Diller (51) studied beech near the border of its range. Correlation of growth with average June temperature, 1913–1933, gives a value of -0.785. His graphs indicate that in 19 years out of the 20, temperature and tree growth moved in opposite directions to each other. Kleine, Potzger and Friesner (119) studied 4 species of oak in Indiana. Their best correlation between temperature and tree growth, 1909–1933, was negative for the period June–August. Little agreement is apparent, for in 10 cases the graphs trend opposite to each other and in 14 cases they trend parallel. Individually also, the years show a mixture of response.

In the South, Coile (38) finds confused responses. Correlation in Georgia gives r a value of -0.80 between June–August temperature and tree growth. In Louisiana, February–May temperature and tree growth gives r as -0.47. In Arkansas, one group shows little agreement with temperature; another gives r as -0.422 with May–July temperature; and a third gives r as $+0.487$ with March–May temperature. Coile ventures no explanation for his diverse results—he appears to appreciate fully the complex nature of growth response.

Fry and White (71) in California emphasize the effect of temperature as it applies to long or short growing seasons. Temperature and soil moisture are inextricably bound together. A dry winter with little snow precedes an early spring, a long growing season, and the production of much wood. MacDougal (137, 138, 139) considers temperature important at the start of cambial activity in the spring and at its cessation in the winter. However, in midsummer, variations of soil moisture commonly determine cambial activity. He calls attention to the case of a maple (137) in which growth stopped by August in spite of high temperature and ample soil moisture.

Mrs. De Geer (50) attempts one of the most ambitious pieces of

correlation to be found, a correlation between clay varves of Sweden and sequoia trees of California. "The curves plotted on the graphs were found to coincide in sequences of striking similarity, grouped at regularly corresponding intervals." In addition, Mrs. De Geer dates the growth layers in logs from ancient ruins in Gottland by means of a graph of growth-layer widths in the sequoias which grew thousands of miles distant. She holds that "tree-rings as well as clay-*varves* can be termed 'self-registering thermographs.'" Even granting the possibility of the future demonstration of such distant correlations, we desire right now much fundamental work in meteorology similar to that of Ångström (6).

On the whole, the effects of temperature on tree growth may be direct or indirect; the effects may be detectable or they may not be. Temperature and tree growth may fluctuate in parallel or opposite fashion. Different months or different month intervals in different localities appear to give the best correspondence, if any exists. Clearly, then, the widths of growth layers give us very little consistent information on temperature conditions. Difficulties arise in attempting to distinguish warm-dry, warm-wet, cold-dry and cold-wet. Which temperature condition is important: average yearly, average seasonal, average monthly, temperature summations, mean temperature, mean maximum or mean minimum? A fair correspondence between tree growth and July temperature, furthermore, does not indicate the temperature conditions for the rest of the year. Then, too, the response to temperature is not present every year, it does not show the same degree of relation when present, and it is not consistently related to the same period. That the results to date can be used to infer past temperature conditions appears unjustified. The results do emphasize the importance of temperature as one of the factors influencing tree growth.

RAINFALL INTERVAL TO WHICH GROWTH CORRESPONDS

Since soil moisture, which becomes the soil solution absorbed by the trees, depends ultimately upon rainfall, it is perhaps natural that one of the early attempts to obtain quick information on past rainfall should have been made by the correlation of tree growth with present rainfall records. An unsuspected difficulty soon confronted those who correlated: to what rainfall period does the tree growth correspond? Obviously, a tree will not record rainfall which does not influence its soil moisture or its nutritive processes.

The rainfall periods to which growth is said to correspond show so much diversity that the summary is worthy of notice:

New England:
  May–July (89).
  April–August (129, 131, 134).
Indiana:
  June–August (119).
  June (51).
Illinois:
  Calendar year (72).
Nebraska:
  Annual (197).
Southeastern United States:
  June of previous year–August of same year as tree growth (173).
Louisiana:
  January–May (38).
Arkansas:
  April–June (38).
Tennessee:
  Water year (previous October–September*) (102).
  Previous October–September* (124).
Northern Washington:
  January–August (125).
Eastern Oregon:
  Previous September–August (116).
  Previous October–September (113).
Northeastern California:
  Previous September–August (46).
  Previous October–September (9).
Western California:
  January–July, or previous November–October (137).
  Previous July–June (138).

* Previous October–September means October of previous year—September of same year as tree growth.

The above list includes simple statements. Actually, workers have found comparisons anything but simple. In New England, Lyon (133, 134) finds no correlation between certain trees and any precipitation period. However, white pine seems very sensitive to water supply of the growing season. Lyon is surprised to note significant correlations with the rainfall of April–August, June, May–July, April–July, April–August, and April–August plus preceding September–November. Coile (38) has nearly the same situation in Georgia where he finds, at Waycross, statistically significant correlations between tree growth and the rainfall of February, January–March, February–April, January–April, and February–May. In Indiana, according to Diller (51), June precipitation has a marked effect on certain woodlands but not on others.

Difficulties attendant upon selecting *one* rainfall interval to which it is supposed the trees respond are well illustrated by Kleine, Potzger and Friesner (119) who give unusually complete data. They obtain very good correspondence with June–August rainfall. However, five out of 24 years have opposite trends. Of these trends opposite to June–August rainfall, four agree with April–May rainfall, and one agrees with the annual rainfall. The writers stress the periods April–May, June–August, and annual. Since

there are 7 cases when the 3 rainfall periods agree with each other in trend, only 7 possible chances exist for the tree growth *not* to agree with one of the three periods. In 10 years out of 24, April–May rainfall does not agree with annual; in 14 out of 24, April–May rainfall does not agree with June–August, and in 10 out of 24, June–August does not agree with annual.

In northern Arizona, Glock (84) finds the rainfall of January, February and April to give the best agreement with tree growth, and yet he speaks (82) of wet and dry "years." Douglass (56) thinks his 1900-year chronology in Arizona gives him fairly close winter rainfall values although his basis for so thinking is statistical correlation of tree growth with more or less distant rainfall records. Jessup (113) used 4 junipers out of a "considerable" number collected and obtains a fairly good agreement for the interval of 1904–1930 between tree growth and the rainfall of the previous October–September taken from weather stations 6 to 45 miles away. Four out of the 27 years show opposite trends. On the basis of other comparisons with rainfall, not to mention the more fundamental aspects of soil moisture and plant physiology, is it safe for Jessup to infer the rainfall of 1760–1900 on the basis of 1904–1930? Antevs (10) shows how such an inference may be in error.

The distribution of rainfall and its effect upon soil moisture in the ground when trees need it are matters of greatest importance and have been emphasized repeatedly (24, 38, 46, 51, 82, 85, 86, 116, 120, 134, 137, 138, 159). Remarks made by Henry (104) and by Davis and Sampson (46) indicate that neither the whole year nor any part thereof furnishes valid information on the rainfall of the remainder. In this connection, Henry refers to the rainfall of the year 1930 in Arkansas which was 96% normal. January, however, had 223% normal, May 200%, June 22%, and July 19%. Here, then, is something to consider when tree growth-rainfall correlations are made, something to consider seriously when interpretations into the past or into the future are contemplated. Such departures from month to month illustrate the hazards in assuming that the rainfall of any one interval indicates the rainfall of any other interval.

The response of tree growth to rainfall, like that to temperature, is not present every year and is not consistently related to the same period.

CORRESPONDENCE OF TREE GROWTH WITH RAINFALL

Previous discussion has eliminated the necessity of a full consideration of the present topic although prevalence of efforts to gain some sort of usable correspondence between tree growth and rainfall makes it imperative to summarize pertinent work for a clear picture of recent trends in what has been called "short-cut" methods. Many workers use the Pearsonian correlation coefficient. Some of these fail to give sufficient data or graphs for an independent analysis. A few students, as Lathe and McCallum (125), present graphs or data for the inspection of their readers. No better practice exists for showing the amount of correspondence between tree growth and rainfall.

Difficulties in the way of good correlations center upon distance to rain gauges and distribution of precipitation. Some workers do not mention the distance to or the location of the weather stations. The following specific difficulties are cited by those making direct tree growth-rainfall comparisons: weather station in the open (3); distance to weather stations (3, 66, 125); particular site factors (3, 24, 89); difference in soil characters (46, 125, 131); distribution of rainfall (23, 46, 89, 125, 134); width of ring as not indicative of volume increase (3, 23, 24, 137, 177); and agreement or its failure among the trees themselves (17, 46, 55, 97, 116, 121, 129, 131, 134). In regard to the last mentioned difficulty, an example will be taken from the work of Lyon because he gives the data. The graphs (129) of two hemlocks, three radii averaged on each, are compared for the interval 1595–1927. "Since both did respond well and in most years in the same way, they can be used for analysis of the relationship of hemlocks in that forest to such a factor as rainfall." Although the small scale of the graphs makes analysis difficult, an interval selected at random, 1751–1780, and counted for trend, gives 12 years parallel trend and 18 opposite. On the basis of the selected interval, one can not, therefore, agree with Lyon. Later work (134) between different species gives no better results. As these trees grew "in shallow soils over and beside a ledge with irregular outcrops," they no doubt emphasize or exaggerate the rainfall factor. Also, it must be noted, they grew beside a rain gauge. "White Pine, Scotch Pine, and Red Oak gave significant, positive correlation coefficients with the rainfall of cer-

tain periods of the year, but Austrian Pine, Norway Spruce, and European Larch showed no consistent agreement with precipitation records. . . ." Surely it is somewhat hazardous to deduce past rainfall conditions until we know a great deal more about tree growth.

Those who detect a direct influence of rainfall upon tree growth either by means of correlation coefficients or by graphs do so over intervals ranging from 4 years to more than 60. Degrees of correspondence vary to a considerable extent. On the whole, a number have had fairly positive results as judged by their statements (38, 51, 58, 72, 82, 84, 89, 97, 102, 113, 116, 119, 124, 125, 129, 163, 197, 201). Salisbury and Jane find excellent graphic correlation over an interval of 4 years between spring-summer rainfall and hazel from the chalk cliffs of Dorset. Fuller bases his correlation on one oak tree and the rainfall 30 miles away. His comparisons are only indifferently fair. Keen obtains complicated results because in some years he thought he detected a lag in rainfall effects and in other years no lag. Lassetter, selecting 25 specimens of pine out of a collection of 640 in Tennessee, obtains a value for r of $+0.53$ to $+0.71$, which is "high considering the unmeasurable conditions affecting tree growth, precipitation, and run-off." Such statement will find plenty of agreement. Lathe and McCallum show graphs which possess remarkable agreement in one case. The average of 194 yellow pines is compared with rainfall of 1915–1929. The three opposite trends are of small amplitude but even so the authors in a refreshingly cautious attitude state that the correspondence may be "perhaps partly fortuitous."

A considerable number of students has obtained results which vary from rather indifferent to negative. The list to be cited includes those who have tried tree growth-rainfall comparisons in one way or another but does not include, with one or two exceptions, a host of botanists and meteorologists whose work suggests to them an entirely different, but more arduous, approach to anatomical response dependent upon physiological processes. Many have vainly sought a direct relation (3, 17, 24, 43, 46, 66, 78, 95, 98, 121, 131, 133, 134 in part, 137, 138, 167, 168, 173, 174, 177, 196).*

* In an article just come to hand, Avery, Creighton and Hock (Amer. Jour. Bot. 27: 825–831, 1940) find "only a very slight positive relationship" between hemlocks in Connecticut and August–February or March–July rainfall.

Chamberlain (26), in remarking on much of the work, says: "The chief result was that the ring of a dry year differs from that of a wet year." Even this does not appear to hold true universally.

Antevs (8, 9, 10) gives such an impartial, thorough and conservative (166) picture of the possible factors, other than amount of rainfall, which can affect the tree growth of individual years, that one is left in no doubt as to the uncertainty attendant upon the derivation of past "wet" and "dry" years. Coile (38) realizes the complex nature of the factors affecting tree growth and states his results in a clear fashion apparently without bias. Also, he realizes that the statistical method has limitations and that it is no substitute for a knowledge of plant physiology. Erlandsson (66) says: "If we try and deduce the annual variations of climate from the sizes of the annual rings, we meet with almost invincible difficulties." He worked in northern Europe where, it may be thought by those who depend upon cross-dating, low temperature and abundant rain complicate or mask growth response.

In this connection reference is made to the cross-dating of Douglass (52, 54) among the sequoia of California and to that of Mrs. De Geer (48, 50) between Swedish glacial varves and California sequoia. Such cross-dating, if valid, does not indicate very great complications induced by low temperature and abundant rain. Fry and White, in their painstaking work (71) in California, bring out the fact that the sequoias of the Sierra Nevada commonly are hindered by too much water and only at remote intervals by too little. Too much water, on the one hand, or intense prolonged drought, on the other, elicit identical responses in the trees. Such responses, by the way, are also noted by Erlandsson (66) in the statements of Melander who says that Finland suffers from famine in years of drought as well as in years of abundant precipitation. Glock (85) calls attention to the situation of the sequoia as regards optimal water supply. On the basis of the work of Fry and White, it seems that Glock should have placed the sequoia slightly above the optimal zone rather than at its lower edge near the minimal zone. Personal observation, as a matter of fact, has tended to confirm the work of Fry and White, for in a half dozen groves inspected, all the trees were confined to basins or valleys where their shallow root systems had access to abundant water. Even some of the roots of so-called ridge trees were followed down to spring water. Another word

of caution seems necessary. Mrs. De Geer (48) assumes that the sequoia roots deeply because it is such a large tree, and bases interpretations on her assumption. MacDougal [Amer. Jour. Bot. **24**: 1–2. 1937], by direct observation, found the root system of a giant sequoia less than 6 feet deep. "No evidence is available . . . to show that the roots of the redwoods ever penetrate deeply."

Detailed analysis to picture intimately the results of tree growth-rainfall comparisons can be made in those cases where data or readable graphs are published. However, only several typical examples of analyses will be given here. Mention already has been made that the most elementary comparison is indicated by parallel or opposite trends in the response of tree growth to rainfall. The prime purpose originally of all correlations seems to have been an effort to establish a basis upon which past rainfall could be derived or future rainfall predicted. Barriers to the effort are formidable: *a*) responses are not consistently parallel or opposite (71); *b*) the factor of difference is not consistent; *c*) a certain response of tree growth to present rainfall records gives no basis for the supposition that past responses were similar in nature or degree (168); *d*) the general attitude of the graph of tree growth in the past is not an infallible indicator of past rainfall (10); *e*) trees apparently respond somewhat to the rainfall of a certain period out of the year and this period may not follow the rainfall régime of the rest of the year (104); *f*) the same trees apparently respond to different rainfall periods in different years (89, 119, 131, 134); *g*) the same trees do not have a consistent response with rainfall of the current growing season or that of the year previous—a mixture of lag and no lag (51, 116, 131); *h*) the distribution of the rainfall within the period may prevent the soil moisture which affects growth from giving an accurate record of the amount of rainfall (23, 46, 89, 125, 134); *i*) only a relatively small percentage of the fluctuations in tree growth are climatic in origin (46, 173, 174).

A simple analysis of trend and of the correspondence of crests and troughs in graphs has been made in all cases where readable graphs are published. A few analyses follow. Lyon (131) compares New England tree growth with 6 different rainfall periods and says ". . . it seems possible to regard hemlock tree growth as a good index of physiological dryness in the area concerned." How

the trend of tree growth agrees with the rainfall comes out as follows:

With rainfall of

April–August	20 parallel,	12 opposite.
Preceding April–August	19	13
Preceding September–November	18	14
Preceding April–November	15	17
April–August + preceding Sept.–Nov.	17	15
June	18	14

The table shows best agreement between tree growth and current rainfall of April–August. All growth responses can be accounted for, of course, on the basis of one period or a combination of them. In a later paper Lyon (134) becomes duly cautious but resorts to a correlation coefficient in spite of a poor correspondence among the different trees.

Goldthwait and Lyon (89) used 27 trees from Vermont and New Hampshire for their rainfall comparisons. They prefer the growing season rainfall of May–July and consider it "the dominant element in the complex of climatic factors." A simple trend of tree growth with rainfall gives:

With

May–July	32 parallel,	33 opposite.
April–July + preceding Sept.–Nov.	38	27

As regards correspondence of the 20 crests in the graphs of tree growth and May–July rainfall, there are:

Practically outright agreement	5 cases.
Tree-growth crest follows rainfall crest by 1 year	3
Tree-growth crest precedes rainfall crest by 1 year	4
Crests in tree growth, none in rainfall	3
Crests in rainfall, none in tree growth	5

As regards the 24 crests in the graphs of tree growth and April–July + preceding September–November rainfall, there are:

Generous outright agreement	9 cases.
Tree-growth crest follows rainfall crest by 1 year	3
Tree-growth crest precedes rainfall crest	0
Crests in tree growth, none in rainfall	4
Crests in rainfall, none in tree growth	8

The results, as stated by the authors, seem somewhat better than the above, but they select for their study 31 "critical" years out of 69 as especially likely to show rainfall response. Thus considered, the results are not encouraging.

Hawley (102) averages 42 specimens of cedar [sic] from Tennessee for the interval 1911–1932. The year 1920 is excluded from her computations because the data are erratic. Her correlation of tree growth with the rainfall of the water year [previous October–September?], original data, gives $r = +0.6949$. For the calendar year, $r = +0.6489$. Calculation of r to 4 decimal places imparts a sense of accuracy that does not exist. If her graphs are compared, they show 14 years of parallel trend and 8 of opposite. A comparison of crests and troughs in original data shows:

CRESTS

Outright agreement	2 cases.
Crests in tree growth, none in rainfall	1
Crests in rainfall, none in tree growth	3
Doubtful crests in rainfall	2

TROUGHS

Outright agreement	3 cases.
Troughs in tree growth, none in rainfall	2
Troughs in rainfall, none in tree growth	2

Miss Hawley says of her smoothed graphs of tree growth and precipitation: "It will be noted" from the figures "of cedar growth, precipitation, and run-off curves, that the crests of smoothed tree growth and precipitation curves fall on the same year. . . ." And yet a comparison of the designated graphs yields:

CRESTS

Outright agreement	2 cases.
Tree-growth crest follows rainfall crest by 1 year	1
Crests in rainfall, none in tree growth	2

TROUGHS

Fairly close agreement	1 case.
Tree-growth trough follows rainfall trough by 1 year	1
Tree-growth trough precedes rainfall trough by 1 year	2
Trough in rainfall, none in tree growth	1

These hardly fall on "the same year." Comparisons between original data of tree growth and runoff give the following trends:

For the same year	12 parallel,	10 opposite.
For one year lag	8	13

Since Miss Hawley gives both correlation coefficients and graphs, her paper permits judgment as to the quality of tree growth-rainfall comparisons. Sampson (168) appears, therefore, to be quite

correct when he calls attention to the misuse of growth-layer widths as a direct record of rainfall.

Keen (116) obtains one of the best correlations in his comparison of ponderosa pine and rainfall of eastern Oregon. His comparison of average departures of ring growth from normal with yearly departures of precipitation [previous September–August] gives $r = +0.50$. A trend analysis of the same gives 32 years parallel, 12 opposite. When Keen adds the rainfall of 6 stations in order to lengthen the record, the trend comes out 39 parallel, 25 opposite. There is no consistency of response here. He says that in most cases crests and troughs coincide. In others, a one-year lag appears. An analysis liberally interpreted gives: 16 cases of year to year correspondence, 9 probable cases of a one-year lag, and 6 cases of isolated crests or troughs. A close study of Keen's graphs shows that the relations are not so simple as the figures indicate—there is a complex intermingling of lag and no lag. For instance, the trend of tree growth for one year corresponds to rainfall of the previous year; then the trend of the second year's tree growth corresponds not to the rainfall of its previous year but to its current year of rainfall. Further, rainfall is even less predictable from tree growth than the figures above indicate. Nor is the case of smoothed graphs or general trend of tree growth relative to mean growth any better. The rainfall of 1874–1889 is above average but tree growth is at or below average. Tree growth may go above average 3 years before rainfall does. Again, tree growth drops from 15 to 45% below average whereas rainfall drops only from 0 to 8% below. Keen compares growth deviations with a 2-year cumulative departure of precipitation and gets $r = +0.82$. "Thus, the tree rings show some effect of water conservation, and, in most cases, a lag of one year in full growth response." The above should be compared with what has already been discussed as well as with the following trend of tree growth with rainfall of 1-year lag: 22 cases parallel and 21 opposite. Although Keen obtains good results, relatively, one can not place confidence in the utility or validity of past rainfall conditions taken directly from widths of growth layers.

Schumacher and Day (173) make a most thorough analysis on statistical grounds, but independent appraisal is difficult. They find only a small part of the variations among growth layers due to what may be climatic causes. "Out of the total sum of squares among

the annual ring widths of the separate groups, only from 3 to 12% is in common annual variation ascribable to fluctuations in climate." By special treatment the percentage may be elevated to 30. Some of their groups were not affected by either amount or distribution of rain; others were affected by amount only; and one group was affected by both. The painstaking work of Schumacher and Day indicates that the possibility of partial influence of rainfall may be determined in a locality for the time rainfall records exist.

The above examples were chosen, first, because the workers give us some basis for independent judgment, and second, because their results are the most encouraging from the standpoint of positive correlation. On the whole, the conclusion seems inevitable that growth-layer widths do not yield directly reliable and consistent records of rainfall quantity for any one uniform period of the year and that they can not be used for the derivation of past or future rainfall by the methods employed. The route from rainfall through soil moisture to the formation of cellulose is too devious, too beset by contributing, interacting factors. One can only speculate as yet on how much the true identification of the annual increment has to do with the discrepancies between graphs of tree growth and rainfall.

In the light of present botanical principles, certain published views border somewhat on the extreme. Unfortunately, many of the claims have a wide popular appeal, and therein resides a heavy responsibility, as Blackwelder cautions (16), for those making the claims. Strictly on the scientific side one example is of great interest to every botanist. The cambium of a certain ponderosa pine near Flagstaff, Arizona, was active in 1902, Schulman says (172), over 1/4000th only of its total area. He of course infers activity of the cambium from the occurrence of the partial growth layer, designated as that of 1902, on the surfaces of 9 transverse sections taken about 6 feet apart along the trunk. "In 1903 about 25,000 times as great a volume of wood was laid down as in 1902" and the "average yearly volume growth in the 1920's was apparently about 80,000 times that for 1902 . . ." This he claims to be one of the "striking illustrations of over-accentuation of drought by the tree-trunk." The rainfall records from Flagstaff, 8 miles to the south, are of interest so far as tree growth-rainfall comparisons are concerned, aside from the extreme volume increment implied. Flagstaff lies south of the San Francisco Peaks whereas the pine grew to the

east. Topographic differences do not exceed many of those where other comparisons have been made. Rainfall is in inches:

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
1901 ..	2.85	8.36	.94	.22	2.27	.02	3.02	1.52	.15	1.90	.09	.14
1902 ..	3.17	1.76	2.72	.37	.69	.16	1.28	6.10	1.12	.00	6.75	1.74
1903 ..	.45	2.62	3.77	3.85	1.39	1.88	3.01	2.26	4.68	1.04	.00	.10

	Annual	Nov.–Apr.	Mar.–Aug.	Jan.–Aug.	Jan.–July	Apr.–May	Apr.–June
1901 ...	21.48	14.54	7.99	19.20	17.68	2.49	2.51
1902 ...	25.86	8.25	7.38	16.25	10.15	1.06	1.22
1903 ...	25.05	19.18	16.16	19.23	16.97	5.24	7.12

The average rainfall, 1920–1929, for April–May was 1.99 inches; for April–June, 2.56. By no conceivable combination of rainfall periods do we remotely see the ratio of 1:25,000. Perhaps the most significant period, so far as the data are concerned, is the rainfall of April–June, and yet this period carries no suggestion of 1:80,000 ratio. To approach even remotely such a ratio we must infer that the tree began growth in 1902 in an almost air-dry soil and that the rainfall of April, May and June did no more than barely maintain the turgor necessary for life processes in the meristematic tissues. One wonders (88), as Antevs (8) did, whether the partial growth layer called 1902 in the above really represents an annual increment. If by some extraordinary chance the annual identification were correct, we have left to us only the strongest of evidence that rainfall measured in a gauge is meaningless as an influence on the width of growth layers.

Entirely on the other side of the picture many will agree with Keen (116) or Lyon (131). Keen says, for instance, that tree-ring patterns "are undoubtedly good indicators of such weather conditions as affect plant growth . . . The width of each annual ring represents a summation and net effect of all the factors influencing tree growth. Thus the tree rings become a better measure of good or poor periods for plant growth than can possibly be obtained through any number of weather-recording instruments." However, the summation in the xylem of the basal trunk probably includes merely a rather rough approximation of all factors bearing on the success of the tree.

CLIMATIC CHANGES AND FOREST MIGRATION

There remains, finally, the consideration of forest migration, the change of amplitude or percentage variation in successive growth

layers, and growth patterns as indicators of climate or climatic changes. If one considers recent work in genetics and ecology (33, 34, 106, 109, 193), he is apt to question the ability of growth layers, either in a single sequence or in an extended sequence, to reveal decided changes in climate. Forests expand or contract, the range of a species shifts in latitude, altitude or continentality—these seem to be the more obvious effects of climatic change. Otherwise, we deal with a field of investigation having to do probably with regional and climatic differentiation of ecotypes and ecospecies (34).

Giddings (77) took some cores from trees 100 miles north of Fairbanks, Alaska, some at Fairbanks, and some 100 miles to the south. The growth layers in the increment cores at the south were easily "datable" on the basis of the cores taken 200 miles to the north. "The timberline record carries down the slopes to the valley bottoms with only a decrease in sensitivity, or rather, a decrease in the number of trees giving a consistent record. The record itself does not change. It is to be concluded from the evidence of timberline trees that climatic conditions have been for several centuries uniform over the whole area from the Yukon River south to the Alaska Range." The statement of Giddings in regard to a decrease in the number of trees giving a consistent record without a change of the record emphasizes the dependence of his climatic inferences, like those of a few others, upon the assumption that only trees which cross-date furnish a basis for climatic interpretations. What, then, do the other trees of his collection record? Also, we may compare the statement on climatic uniformity with the work of Griggs (90, 91, 92) who finds good evidence for the migration of Alaskan forest, probably under climatic stimulus. It is, on the whole, quite clear that decided changes of climate induce changes of morphology and of internal structure, as indicated by observations and transplant experiments (33, 34, 36, 106). Furthermore, climatic changes of any consequence, however induced, bring about extensive forest migrations, so well illustrated by the Cenozoic floras of western North America, as clarified by Chaney (27, 28, 29, 30). In either case, change in the organism or migration of the original, decided swings of climate are not necessarily recorded in the amount of xylem formed.

Minor variations in climate, or better in rainfall, have a somewhat greater opportunity of being detectable in growth layers. Some

hope does exist of obtaining an indirect hint of minor rainfall changes by computing the degree of change in width of successive growth layers on a sequence (82, 85). Fluctuations in degree or amplitude of change may be found to parallel in some respects the well known fact (148, 202) that "the smaller the average rainfall the greater is the variation from year to year" (104). Thus, an increase of degree of change in tree growth means a decrease of rainfall for the interval concerned. A fair test of the method must of course be made in a region where and when the identification of the annual increment is an assured fact.

In a somewhat different way, the work of Antevs (10) in northeastern California and of Coile (38) in southern Louisiana must be considered when variations in growth rate from a mean are taken directly to indicate variations in rainfall. Coile notes a decrease in growth rate for 1918–1933. Since the rainfall did not decrease in trend, Coile concludes the decrease in tree growth was due to something beside cyclic change of climate. Growth-layer widths relative to a mean, individually or collectively, give neither a dependable, quantitative estimate of rainfall nor a safe indication of the rainfall over a period of years.

GROWTH PATTERNS

If the principles of Blackman (15) regarding optima and limiting factors apply to plant growth, then the variable amounts of xylem formed might be expected to depend somewhat upon those principles. Glock (82, 85, 87) made use of a minimal, a maximal, and an optimal zone in an attempt to explain direct, inverse, and no response of tree growth to fluctuations of the moisture factor; to explain short, long, and no cycles in tree growth; and to account for the localization of cross-dating in one type of environment. Fry and White (71) bring out clearly the relation of sequoia growth to excess water and to a short growing season. Shreve also describes (176) plant growth under high rainfall. Many others have made at least partial reference to factors present in below, above, or optimum amounts (38, 66, 96, 98, 111, 116, 119, 126, 127, 129, 131, 136, 137, 138, 168).

Continued attempts to correlate growth-layer thicknesses to hundredths of a millimeter with rain-gauge measurements to hundredths of an inch, and the insistence that growth-layer widths can be substi-

tuted for rainfall records, have perplexed many botanists and meteorologists. To avoid these things, Glock (86, 87) attempts to gain a generalized picture of the rainfall régime through the influence of soil moisture on the growth-layer pattern. Much fundamental work already done furnishes a background to the study of growth patterns (3, 13, 18, 20, 21, 23, 24, 37, 38, 40, 41, 119, 137, 138, 160, 195). Dependence is placed (86, 87) upon the pattern of early and late wood formed by soil-moisture fluctuations in relation to the wilting coefficient rather than upon the quantity of xylem manufactured, as measured in linear dimension. The type of rainfall is indicated in so far as it influences soil-moisture replenishment in time and amount. On the whole, type of rainfall gives a general idea of the average amount of rainfall (104, 148, 202). The method admittedly is somewhat indirect and lacks that precision in revealing amounts of rainfall so much sought after the past few years, but it is believed to represent the safe limit of interpretative possibilities based upon present soil-moisture and detailed anatomical studies. Furthermore, Glock depends upon the average trees common to and characteristic of a region rather than upon those so placed locally as to exaggerate the effects of runoff, evaporation, exposure, or soil deficiency for the region. In a very brief and preliminary fashion, the method of growth patterns was applied (45) in 1939 to Upper Triassic wood from Arizona.

Three characteristic and distinct types of growth-layer patterns and rainfall régimes have been briefly described (86, 87) to date: the California [central and south-central Sierra Nevada], the West-Texas, and the Arizona types. The California type is marked by simplicity and uniformity. Ordinarily, when the trees begin to grow in the spring the soil away from streams has all the moisture it will receive during that growing season. This is the region of winter rains. In contrast, the West-Texas type is marked by complexity and variability of pattern. The trees at the initiation of cambial activity seldom have all the moisture they will receive before the growing season ends. This is the region of dominant summer rains in which the brief rainy spells are separated by intense intra-seasonal droughts. Here the annual increments commonly contain more than one growth layer (88). The Arizona pattern holds much interest because it appears to be a combination of the California and West-Texas types. That is to say, groups of growth layers typical

of the California pattern alternate with groups typical of West-Texas.

The situation is more easily visualized by noting that in northern Arizona the pattern comes more and more to resemble the West-Texas with decreasing elevation whereas it increasingly resembles the California pattern with increasing elevation. From a regional standpoint, the West-Texas pattern extends westward at low elevations and the California extends eastward at high. The Arizona pattern characterizes the lower portion of the ponderosa pine belt which, together with the piñon-juniper belt, forms the forest border. The rainfall régime of Arizona is marked by a double rainy season, one in the winter and the other in summer. According to Pearson (152), "records in the western yellow pine have shown that for depths of 12 inches or less, complete or nearly complete exhaustion of growth water may usually be expected in the latter part of June." The upper 12 to 18 inches of forest soil are very probably critical (198). As rainfall to replenish soil moisture varies in amount and in distribution, the soil-moisture régime, pictured by Pearson, will move up or down in elevation. And thus, the West-Texas pattern will move upward in time of drought or poor distribution of rainfall, and the California pattern will move downward in time of abundant rainfall or of equable distribution. Hence, the alternation of pattern characteristics in the Arizona type.

Many statements have been made to the effect that a tree carries its life story within its trunk (173, 174). The statement is the more true if the whole trunk actually were examined. However, it appears quite likely that the trunk, especially at the base, carries a rather incomplete résume, as it were, of the chief episodes in the life history of a tree—a rather crude summation when the entire tree, branches, bole and roots are taken into account. In fact, there exists ample reason (5, 7, 8, 12, 24, 67, 69, 70, 88, 94, 111, 137, 138, 158, 188, 199) for thinking that the most complete story, the most complete record of environmental influence, climate included, resides in the branches.

ASSUMPTIONS

A strict analysis of certain recent publications and the work upon which they are based brings the analyst inevitably to the conclusion that several assumptions have formed the ground work for interpretation. They are here brought together:

1) Any growth layer, or partial growth layer, sharply defined on its radial boundary, necessarily constitutes an annual increment.

2) The maximum number of sharply bounded growth layers, no matter how complete the sheath covering the entire tree or how fragmentary as a partial sheath, constitutes the true number of years of growth.

3) Correlation of growth layers among different trees [so-called cross-dating] proves their annual identity.

4) Cross-dating of growth layers among different trees forms the only basis upon which climatic interpretations can be made from growth layers.

5) Variations in the widths of growth layers in correlated sequences correspond to variations in rainfall with sufficient accuracy to permit the substitution of the one for the other in time or in space.

6) A thick "ring" indicates a wet year and a narrow "ring" a dry year.

7) It is legitimate to make general comparisons between growth-layer widths and rainfall 200 or more miles away and to draw detailed conclusions therefrom.

8) "Dating" can be accomplished on the basis of growth-layer thicknesses thousands of miles away.

9) The width of a growth layer measured on one or several radii yields an accurate index of the precipitation caught by an adjacent gauge.

10) Growth layers record permanent changes in climate, or the larger climatic variations.

11) The Pearsonian method of correlation is a legitimate one for the comparison of tree-growth and rainfall variations.

12) A moderate correlation coefficient between tree growth and rainfall of the present day serves as an adequate basis for the derivation of the rainfall from growth layers which grew long before weather records were taken.

13) Cycles in growth-layer width parallel or duplicate cycles in rainfall.

The botanist will immediately recognize that these assumptions give rise to many implications in regard to complexity of growth factors, physiological functions, cambial activity, soil-moisture relations, sap concentration, food reserves, growth intensity periods, and activator substances.

CONCLUSION

Publications from 1935 onward have been of chief concern in the present connection. The desired purpose, so far as possible, has been to make an impartial analysis, and thereby give the reader an idea of the nature and content of recent work, the quality of the work, and the relation of that work to botanical research in general. Three points have influenced the analysis: *a*) material available, *b*) adherence to botanical principles, and *c*) the training and experience of the man responsible for the publication. Undoubtedly, there are foreign works which were not available. Much gratitude is due Dr. R. A. Studhalter who critically read the entire manuscript.

Many investigators (9, 38, 46, 66, 71, 89, 116, 125, 131, 173) have made relatively impartial studies of the tree growth-rainfall relationship, studies which indicate their realization of the difficulties, of the uncertainties inherent in the work, and of the lack of definite objective aside from that of simple relationship for the years concerned.

Words of caution have been written from time to time concerning speculations freely made upon assumptions (3, 8, 10, 14, 17, 23, 24, 43, 67, 71, 111, 117, 136, 137, 165, 166, 167, 168). However, constructive suggestions concerning the problems of tree growth have not been wanting—suggestions that stress the need of basic research (3, 18, 20, 23, 24, 46, 82, 85, 86, 110, 111, 120, 131, 137, 138, 167, 187, 196). Much work fundamental to future investigations has been done; it forms a background of no more than the minimum and thus forms a point of departure (3, 4, 11, 12, 15, 22, 23, 24, 25, 28, 34, 36, 37, 44, 65, 74, 93, 105, 106, 110, 111, 112, 118, 128, 137, 138, 138*b*, 139, 143, 147, 152, 153, 156, 157, 158, 159, 162, 164, 167, 178, 179, 184, 190, 193, 195, 198, 200, 203).

The consensus of opinion among botanists, meteorologists, climatologists, and others shapes itself rather definitely. Correlations, if legitimate, may corroborate suspected relationships but they neither reveal cause nor constitute a basis for interpretation beyond the samples participating in the calculations. It seems abundantly clear, first, that rainfall and temperature are of great importance to tree growth, second, that under a certain combination of interacting factors in certain localities rainfall and temperature have such an influence on physiological processes as to bring about a degree of similarity at times in the fluctuations of tree growth and rainfall or

temperature, and third, that correlations, even were they of high degree, do not permit the derivation of past or future rainfall.

The derivation of climate from tree growth should be sought, apparently, by other means than by direct quantitative attempts to base amount of rainfall on width of growth layers although at times this, in a measure, can be done. Without doubt, "the methods of an ecologically minded botanist" applied to tree growth through physiological function and its dependent anatomical response should supplant the more simple, the more obvious but less fruitful and less valid methods of direct correlations. An understanding of plant physiology and anatomy brought about by judicious experimentation under the strict discipline of the botanist may ultimately reveal the criteria by which growth layers and their cellular structure will yield a picture of the soil-moisture régime and perhaps thereby indirectly a picture of rainfall type.

Bibliography

1. Abbe, Cleveland. [See J. Keuchler.]
2. Abbot, C. G. Cycles in tree-ring widths. Smith. Misc. Coll. **95** (19): 1–5. 1936.
3. Adams, W. R. Studies in tolerance of New England forest trees. XII. Effect of thinning in plantations on some of the physical factors of the site and on the development of young northern white pine (*Pinus strobus* L.) and Scotch pine (*Pinus silvestris* L.). Vermont Agr. Exp. Sta. Bull. 390: 1–156. 1935.
4. Allee, W. C., and Park, T. Concerning ecological principles. Science **89**: 166–169. 1939.
5. Andrews, S. R., and Gill, L. S. Determining the time branches on living trees have been dead. Jour. For. **37**: 930–935. 1939.
    a. Flury, P. Über Alterbestimmung mittels Jahresringzählung. Allg. Forst. Jagdz. **100**: 352–355. 1924.
    b. Rubner, K. Das Hungern des Kambiums und das Aussetzen der Jahrringe. Naturw. Zeits. Forst. Landw. **8**: 212–262. 1910.
6. Ångström, A. Teleconnections of climatic changes in present time. Geog. Annaler **17**: 242–258. 1935.
7. Antevs, E. The Big Tree as a climatic measure. Carnegie Inst. Wash. Pub. 352: 115–153. 1925.
8. ———. The tree ring as a scientific tool. A review of "Principles and methods of tree-ring analysis" by Waldo S. Glock. Geog. Rev. **28**: 518–520. 1938.
9. ———. Rainfall and tree growth in the Great Basin. Carnegie Inst. Wash. Pub. 469: 1–97. 1938. (Amer. Geog. Soc. Special Pub. 21.)
10. ———. Precipitation and water supply in the Sierra Nevada, California. Bull. Amer. Met. Soc. **20**: 89–91. 1939.
11. Ashby, E. Statistical ecology. Bot. Rev. **2**: 221–235. 1936.
12. Avery, G. S., Jr., Burkholder, P. R., and Creighton, H. B. Production and distribution of growth hormones in shoots of *Aesculus* and *Malus*, and its probable rôle in stimulating cambial activity. Amer. Jour. Bot. **24**: 51–58. 1937.
13. Baldwin, H. I. The period of height growth in some northeastern conifers. Ecology **12**: 665–689. 1931.

a. Burger, H. Untersuchungen über das Hohenwachstum verschiedener Holzarten. Mitt. Schweiz. Centralanstalt Forst. Vers. 14: 1–158. 1926.
b. Dostal, R. Über die Sommerperiodizität bei *Quercus* und *Fagus*. Ber. Deut. Bot. Ges. 45: 436–446. 1927.
c. Klebs, G. Über das Treiben der einheimischen Bäume speziell der Buche. Diss. Heidelberg. 1914. [Review in For. Quart. 14: 83. 1916.]
d. Nakashima, H. Über den Einfluss meteorologischen Faktoren auf den Baumzuwachs. II. Jour. Fac. Agr. Hokkaido Imp. Univ. 22: 301–327. 1929.
e. Tolsky, A. P. Trans. For. Exp. St. Petersburg 47. 1913. [Abstract in For. Quart. 12: 277–278. 1914.]

14. Barnett, R. J. Tree rings as recorders of weather cycles. Bull. Amer. Met. Soc. 16: 44. 1935.
15. Blackman, F. F. Optima and limiting factors. Ann. Bot. 19: 281–295. 1905.
16. Blackwelder, Eliot. Science and human prospects. Bull. Geol. Soc. Amer. 52: 295–312. 1941.
17. Bonner, F. E. Climatic cycles in the West: no direct relation found between sun spots, tree rings, and rainfall. Civil Eng. 5: 343–346. 1935.
a. Jarvis, C. S. Rainfall characteristics and their relation to soils and runoff. Trans. Amer. Soc. Civil Eng. 95: 398. 1931.
18. Bowman, I. The land of your possession. Science 82: 285–293. 1935.
19. ———. Our expanding and contracting "Desert." Geog. Rev. 25: 43–61. 1935.
20. Brady, L. F. An effect of starvation on pine trees. Tree-Ring Bull. 5: 20–23. 1939.
21. Bunger, M. T., and Thomson, H. J. Root development as a factor in success or failure of windbreak trees in the southern High Plains. Jour. For. 36: 790–803. 1938.
22. Burkholder, P. R. The rôle of light in the life of plants. II. The influence of light upon growth and differentiation. Bot. Rev. 2: 1–52. 97–172. 1936.
23. Burns, George P. Studies in tolerance of New England forest trees. IX. Rainfall and width of annual rings in Vermont forests. Vermont Agr. Exp. Sta. Bull. 298: 1–24. 1929.
24. ———. Studies in tolerance of New England forest trees. XIII. The effect of root development on height and diameter growth. Vermont Agr. Exp. Sta. Bull. 422: 1–29. 1937.
25. Carlson, A. J. Science versus life. Science 93: 93–100. 1941.
26. Chamberlain, C. J. The gymnosperms. Bot. Rev. 1: 183–209. 1935.
27. Chaney, Ralph W. The succession and distribution of Cenozoic floras around the northern Pacific Basin. Essays in Geobotany in Honor of William Albert Setchell, 55–85. 1936.
28. ———. Paleoecological interpretations of Cenozoic plants in western North America. Bot. Rev. 4:. 371–396. 1938.
29. ———. Ancient forest of Oregon: a study of earth history in western America. Cooperation in Research. Carnegie Inst. Wash. Pub. 501: 631–648. 1938.
30. ———. Tertiary forests and continental history. Bull. Geol. Soc. Amer. 51: 460–488. 1940.
31. Chapman, H. H. Why aborigines left Chaco Canyon. Amer. For. 46: 98. March 1940.
32. ———. Comments. Jour. For. 38: 968. 1940.
33. Clausen, Jens, Keck, D. D., and Hiesey, W. M. Experimental Taxonomy. Carnegie Inst. Wash. Yearbook 37: 218–222. 1938.
34. ———, ———, ———. Experimental studies on the na-

ture of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Wash. Pub. 520: 1–452. 1940.

35. Clements, F. E. Climatic cycles and human populations in the Great Plains. Carnegie Inst. Wash. Suppl. Pub. 43: 1–19. 1938.
36. ———, and Clements, E. S. Climax, succession, and conservation. Carnegie Inst. Wash. Yearbook 37: 233. 1938.
37. ———, Long, F. L., and Martin, E. V. Factor and function in adaptation. Carnegie Inst. Wash. Yearbook 37: 229–233. 1938.
38. Coile, T. S. The effect of rainfall and temperature on the annual radial growth of pine in the southern United States. Ecol. Mono. **6**: 533–562. 1936.
39. Cooperrider, C. K., and Sykes, G. G. The relationship of stream flow to precipitation on the Salt River watershed above Roosevelt Dam. Ariz. Agr. Exp. Tech. Bull. 76: 1–69. 1938.
40. Cottle, H. J. Studies in the vegetation of southern Texas. Ecology **12**: 105–155. 1931.
41. ———. Vegetations on the north and south slopes of mountains in southwest Texas. Ecology **13**: 121–134. 1932.
42. Craighead, F. C. Some effects of artificial defoliation on pine and larch. Jour. For. **38**: 885–888. 1940.
43. Crandall, Lynn. Tree rings and runoff in the Northwest. Civil Eng. **5**: 638–639. 1935.
44. Croxton, F. E., and Cowden, D. J. Applied general statistics. 678–679, 804–805, 810, 822. 1940.
45. Daugherty, Lyman H. The Upper Triassic flora of Arizona. With a discussion of its geologic occurrence by Howard R. Stagner. Carnegie Inst. Wash. Pub. 526: 30, 33, 35. 1941.
46. Davis, W. E., and Sampson, A. W. Experiment in correlation of tree-growth rings and precipitation-cycles. Trans. Amer. Geophys. Union: 493–496. 1936.
47. De Geer, E. H. Prehistoric bulwark in Gottland biochronologically dated. Geog. Annaler **17**: 501–531. 1935.
48. ———. Teleconnection of geochronology and historic time. Int. Geol. Cong., Rep. XVI session, U.S.A. 1933. **1**: 203–211. 1936.
49. ———. Jahresringe und Jahrestemperatur. Geog. Annaler **18**: 277–297. 1936.
50. ———. Biochronology. Scot. Geog. Mag. **52**: 145–147. 1936.
51. Diller, Oliver D. The relation of temperature and precipitation to the growth of beech in northern Indiana. Ecology **16**: 72–81. 1935.
52. Douglass, A. E. Climatological researches. Carnegie Inst. Wash. Yearbook 30: 278–280. 1931.
53. ———. Climatological researches. Carnegie Inst. Wash. Yearbook 31: 217–219. 1932.
54. ———. Climatological researches. Carnegie Inst. Wash. Yearbook 32: 208–211. 1933.
55. ———. Climatological researches. Carnegie Inst. Wash. Yearbook 33: 196–198. 1934.
56. ———. Climatological researches. Carnegie Inst. Wash. Yearbook 34: 215–219. 1935.
57. ———. Climatological researches. Carnegie Inst. Wash. Yearbook 35: 227–230. 1936.
58. ———. Climatic cycles and tree growth. Volume III. A study of cycles. Carnegie Inst. Wash. Pub. 289: 1–171. 1936.
59. ———. Tree-rings and chronology. Univ. Ariz. Bull. **8**: 1–36. 1937.
60. ———. Climatological research. Carnegie Inst. Wash. Yearbook 36: 228. 1937.
61. ———. Forward. *In* Principles and methods of tree-ring analysis, by Waldo S. Glock. Carnegie Inst. Wash. Pub. 486: VII–VIII. 1937.

62. ————. Typical site of trees producing best crossdating. Tree-Ring Bull. 6: 10–11. 1939.
63. ————. Compression wood and recent chronology in Mesa Verde firs. Tree-Ring Bull. 6: 23–24. 1940.
64. ————. Editorial: a bibliography. Tree-Ring Bull. 6: 26–27. 1940.
65. du Buy, H. G. New research on growth and tropisms. Chron. Bot. 6: 80–83. 1940.
66. Erlandsson, S. Dendrochronological studies. Geog. Annaler 18. 1936.
   a. Broocman, R. En Fulständig Swensk Hus-Håldsbok. Norrköping. 1736.
   b. *v.* Linné, C. Öländska och Gothländska Resa. (Stockholm och Upsala 1745.) Westgöta Resa. Stockholm. 1747.
   c. Pokorny, A. Eine Methode um den meteorologischen Coefficienten des jährlichen Holzzuwachses der Dicotyledonenstamme zu ermitteln. Bot. Zeit. 26: 1869.
   d. Reventlow, C. D. F. Grundsätze und Regeln für der Zwickmässigen Betrieb der Forsten. Posthum. edition by A. H. Grøn. Kopenhagen. 1934.
   e. Rudenschöld, U. Rön om Furuträdens Ålder uti Finnland. K. Sv. Vetakad. Handl. Band VII. 1746.
   f. Wastenson, A. Linné als Biochronolog. Sv. Linné-Sällsk. Arsskr. Bd. X. 1927.
67. Evenden, J. C. Effects of defoliation by the pine butterfly upon ponderosa pine. Jour. For. 38: 949–955. 1940.
   a. Craighead, F. C. Studies on the spruce budworm. Dept. Agr., Dom. Canada, Bull. 37. 1924.
   b. Graham, S. A. The effects of the defoliation of tamarack. Jour. For. 29: 199–206. 1931.
68. Fisher, R. A. Statistical methods for research workers. 7 ed. 1–356. 1938.
69. Fritz, E. Growth of redwood trees left after selective logging. The Timberman 39 (8): 14–16, 53–55. 1938.
70. ————. Problems in dating rings of California coast redwood. Tree-Ring Bull. 6: 19–21. 1940.
71. Fry, W., and White, J. R. Big Trees. 37–44. 1938.
72. Fuller. G. D. Growth rings of the oak as related to precipitation in Illinois. Trans. Ill. Acad. Sci. 31: 102–104. 1938.
73. Furras, R. W. Tree rings. Ariz. Weekly Star. Tucson: 4. 22 Dec. 1887.
74. Gast, P. R. Studies on the development of conifers in raw humus. III. The growth of Scots pine (*P. silvestris* L.) seedlings in pot culture of different soils under varied radiation intensities. Medl. Statens Skogsförsöksanstalt 29: 587–682. 1937. [Review in Ecology 18: 535–538. 1937.]
75. Geiser, S. W. Naturalists of the frontier. 1937.
76. ————. A century of scientific exploration in Texas. Part I*b*: 1820–1880. Field and Lab. 7: 29–52. 1939.
77. Giddings, Jr., J. L. Recent tree-ring work in Alaska. Tree-Ring Bull. 5: 16. 1938.
78. Girand, John. Tree ring studies. Bull. Amer. Met. Soc. 16: 24–26. 1935.
79. Glock, Waldo S. *In* Climatological researches, by A. E. Douglass. Carnegie Inst. Wash. Yearbook 32: 209. 1933.
80. ————. *In* Climatological researches, by A. E. Douglass. Carnegie Inst. Wash. Yearbook 33: 196–198. 1934.
81. ————. Observations on the western Juniper. Madroño 4: 21–28. 1937.

82. ———. Principles and methods of tree-ring analysis. Carnegie Inst. Wash. Pub. 486: 1–100. 1937.
83. ———. Tree-ring dating: factors pertaining to accuracy. Tree-Ring Bull. **4**: 6–8. 1938.
84. ———. *In* Historical climatology, by A. E. Douglass. Carnegie Inst. Wash. Yearbook 37: 235. 1938.
85. ———. Tree-growth indices to past climates. Pan-Amer. Geol. 70: 135–144. 1938.
86. ———. Rain-fall types and tree-growth patterns as indices to past climates. Pan-Amer. Geol. **74**: 290–296. 1940.
87. ———. The response of trees to climate. Proc. VI Pacific Sci. Congr., 1939. 3: 617–619. 1941.
88. ———, Reed, Sr., E. L. Multiple growth layers in the annual increments of certain trees at Lubbock, Texas. Science **91**: 98–99. 1940.
89. Goldthwait, L., and Lyon, C. J. Secondary growth of white pine in relation to its water supply. Ecology **18**: 406–415. 1937.
90. Griggs, Robert F. The problem of arctic vegetation. Jour. Wash. Acad. Sci. **24**: 153–175. 1934.
91. ———. The edge of the forest in Alaska and the reasons for its position. Ecology **15**: 80–96. 1934.
92. ———. Timberlines as indicators of climatic trends. Science **85**: 251–255. 1937.
93. Haasis, F. W. Diametral changes in tree trunks. Carnegie Inst. Wash. Pub. 450: 1–103. 1934.
94. Haberlandt, G. Physiologische Pflanzenanatomie. 4th Ed. 1909. Transl. by M. Drummond. 1914.
95. Hale, J. D., and Prince, J. B. Density and rate of growth in the spruces and balsam fir of eastern Canada. Dept. Mines and resources; Lands, Parks, and Forests Branch, Dom. For. Ser. Bull. **94**: 1–43. 1940. [Review in Jour. For. **38**: 904. 1940.]
96. Harding, S. T. Changes in lake levels in Great Basin area. Civil Eng. **5**: 87–90. 1935.
97. Hardman, George. The relationship between tree-growths and stream-runoff in the Truckee River basin, California-Nevada. Trans. Amer. Geophys. Union, Nat. Res. Council, 1936, pt. 2: 491–493. 1936.
98. Hardman, George, and Reil, O. E. The relationship between tree growth and stream run-off in Truckee River basin, California-Nevada. Univ. Nev. Agr. Exp. Sta. Bull. 141: 1–38. 1936.
99. Haury, E. W. Ancient American droughts. Literary Digest **18**. 2 March 1935.
100. ———. Tree-rings—the archaeologist's timepiece. Amer. Antiquity **1**: 98–108. 1935.
101. Hawley, F. Significance of the dated prehistory of Chetro Ketl, Chaco Canyon, New Mexico. Univ. New Mexico Mono. Ser. 1 (1). 1934.
102. ———. Relationship of southern cedar growth to precipitation and run-off. Ecology **18**: 398–405. 1937.
103. ———, and Clark, N. M. Trees do not lie! Amer. For. **46**: 66–68, 95–96. 1940. [Abstracted in Readers Digest **36** (216): 102–104. 1940.]
104. Henry, A. J. The calendar year as a time unit in drought statistics. Mon. Weather Rev. **59**: 150–154. 1931.
105. Heyward, Frank. The root system of longleaf pine on deep sands of western Florida. Ecology **14**: 136–148. 1933.
106. Hiesey, William. Environmental influence and transplant experiments. Bot. Rev. **6**: 181–203. 1940.
107. Holmes, O. W. The aristocrat of the breakfast table. 1892.
108. Humboldt, A. von. Aspects of nature. Transl. by Mrs. Sabine. 1849.

109. HUNTINGTON, E. (Climatic changes). Carnegie Inst. Wash. Yearbook 14: 93–97. 1915.
110. HURSH, C. R., AND CONNAUGHTON, C. A. Effects of forests upon local climate. Jour. For. 36: 864–866. 1938.
111. JACCARD, P. Neue Untersuchungen über die Ursachen des Dickenwachstums der Bäume. Div. Silvics, U. S. For. Ser. Transl. 176: 1–39. 1935.
112. JENNY, H., AND OVERSTREET, R. Nutrients for plant roots from dry soil. Science 92, suppl.: 9, 11. 13 Dec. 1940.
113. JESSUP, L. T. Precipitation and tree growth in the Harney Basin, Oregon. Geog. Rev. 25: 310–312. 1935.
114. JONES, MARCUS E. Douglas and annual rings. Contr. West. Bot. 18: 11. 1933–1935.
115. KARRAKER, P. E., AND BORTNER, C. E. Availability of soil moisture, particularly as affected by depth, in the soil of the Kentucky Experiment Station farm at Lexington. Jour. Amer. Soc. Agron. 31: 653–660. 1939. [From Biol. Abs.]
116. KEEN, F. P. Climatic cycles in east Oregon as indicated by tree rings. Mon. Weath. Rev. 65: 175–188. 1937.
117. KEUCHLER, J. (=J. Kuechler). Notes by the Editor on tree-growth work of J. Keuchler as reported by W. W. Haupt. Mon. Weath. Rev. 21: 331–332. 1893. [Originally published in the San Antonio, Texas, "Zeitung." 1859.]
118. KIENHOLZ, R. Effect of environmental factors on the wood structure of lodgepole pine, *Pinus contorta* Loudon. Ecology 12: 354–379. 1931.
119. KLEINE, A., POTZGER, J. E., AND FRIESNER, R. C. The effect of precipitation and temperature on annular-ring growth in four species of *Quercus*. Butler Univ. Bot. Studies 3 (14): 199–205. 1936.
120. KOEHLER, A. More about tree-rings and weather. U. S. For. Ser. Bull. 3–4. Dec. 1937.
121. KOLMODIN, G. Väderlekens inflytande på tallens diameter-telväxt. U. S. For. Ser. Transl. 259. 1936.
122. KROL, O. Adatok a növenyek "volságos idöszak"—ánok kérdéséhez (The "critical period" of plants). Erdiszeti Lapok. 78 (4): 382–393. 1939. [From Biol. Abs.]
123. KUECHLER, J. [See J. Keuchler. Geiser uses the original name of "Küchler."]
124. LASSETTER, ROY. The value of tree-ring analysis in engineering. Tree-Ring Bull. 5: 13–15. 1938.
125. LATHE, F. E., AND MCCALLUM, A. W. Effect of sulphur dioxide on vegetation. Chap. VII. The effect of sulphur dioxide on the diameter measurement of conifers. Nat. Res. Council Canada 815: 174–206. 1939.
126. LAWRENCE, D. B. Submerged forest of the Columbia River gorge. Geog. Rev. 26: 581–592. 1936.
127. LUKKOLA, O. J. Nölkävuosien suonkuivansten tuloksia (Results of moor drainage during famine years). Comm. Inst. Forest. Fenniae 24 (3): 1–160. 1937 (1938). [From Biol. Abs.]
128. LUNDEGARDH, H. Environment and plant development. [Transl. by E. Ashby from 2d German Ed. 1931.]
129. LYON, C. J. Rainfall and hemlock growth in New Hampshire. Jour. For. 33: 162–168. 1935.
130. ———. Tree rings in New England. Science 81: 340–341. 1935.
131. ———. Tree ring width as an index of physiological dryness in New England. Ecology 17: 457–478. 1936.
132. ———. The first dating of preserved wood in New England. Science 90: 419–420. 1939.
133. ———. Tree rings fallible as records of weather. Sci. News Let. 13: 29–30. Jan. 1940.

134. ————. Tree growth beside a rain gauge and thermometer. Ecology **21**: 425–437. 1940.
135. MacDougal, D. T. Influence of aridity upon the evolutionary development of plants. Plant World **12**: 217–231. 1909.
136. ————. Relation of leaf-surface to wood formation in pine trees. Proc. Amer. Phil. Soc. **72**: 325–332. 1933.
137. ————. Studies in tree growth by the dendrographic method. Carnegie Inst. Wash. Pub. 462: 1–256. 1936.
138. ————. Tree growth. Chron. Bot. **4**: 1–240. 1938.
 a. Fabricius, L. Untersuchungen uber den Stärke- und Fettgehalt der Fichte auf der oberbayerische Hochebene. Naturw. Zeit. Land- u. Forstwiss. 3: 137–176. 1905.
 b. Ishibe, O. The seasonal changes in starch and fat reserves of some woody plants. Mem. College of Sci. Kyoto Imp. Univ., Ser. B. **11** (1). 1935.
139. ————. Autumnal increase in diameter of tree trunks. Chron. Bot. **4**: 489–490. 1938.
140. McGrath, Thomas. Comments on climatic cycles. Civil Eng. **5**: 709. 1935.
141. McMurrick, J. Playfair. Leonardo da Vinci, the anatomist. Carnegie Inst. Wash. Pub. 411: 247. 1930.
142. Magistrad, O. C., and Breazeale, J. F. Plant and soil relations at and below the wilting percentage. Ariz. Agr. Exp. Sta. Tech. Bull. 25: 1–36. 1929.
143. Mallery, T. D. Changes in the osmotic value of the expressed sap of leaves and small twigs of *Larrea tridentata* as influenced by environmental conditions. Ecol. Mono. **5**: 1–35. 1935.
144. Marshall, G. E., and Troth, M. S. The reciprocal of rainfall and temperature as it affects the apple crop. Trans. Ind. Hort. Soc. **1937**: 123–129. 1937 (1938).
145. Martin, W. E. Some effects of cultural practices upon tree composition, yield and quality of Marsh grapefruit in Arizona. Proc. Amer. Soc. Hort. Sci. **37**: 68–75. 1939.
146. Mercykowski, Dimitri. The romance of Leonardo da Vinci. The Modern Library, book 10, Chap. 9. 1928. [Transl. by B. G. Guerney. 1930. Garden City Pub. Co.]
147. Meyer, B. S., and Anderson, D. B. Plant physiology. 1939.
148. Mixer, C. A. The rainfall year. Bull. Amer. Met. Soc. **15**: 22–23. 1934.
149. Muller, C. H. Relations of the vegetation and climatic types in Nuevo Leon, Mexico. Amer. Mid. Nat. **21**: 687–729. 1939.
150. Paul, B. H., and Marts, R. O. Controlling the proportion of summer wood in longleaf pine. Jour. For. **29**: 784–796. 1931.
151. Pearson, G. A. The growing season of western yellow pine. Jour. Agr. Res. **29**: 203–204. 1924.
152. ————. Forest types in the Southwest as determined by climate and soil. U. S. Dept. Agr. Tech. Bull. 247: 1–143. 1931.
153. ————. Factors influencing the growth of trees. Carnegie Inst. Wash. Pub. 486: 65–68. 1937.
154. Pessin, L. J. Annual ring formation in *Pinus palustris* seedlings. Amer. Jour. Bot. **21**: 599–601. 1934.
155. Pillow, M. Y., and Luxford, R. F. Structure, occurrence and properties of compression wood. U. S. Dept. Agr. Tech. Bull. 546: 1–32. 1937.
 a. Johnson, B., and Hovey, R. W. Determination of cellulose in wood. Jour. Soc. Chem. Ind. Trans. **37**: 132T–137T. 1918.
156. Potzger, J. E. Microclimate and a notable case of its influence on a ridge in central Indiana. Ecology **20**: 29–37. 1939.
157. ————. Microclimate, evaporation stress, and epiphytic mosses. Bryologist **42**: 53–61. 1939.

158. PRIESTLEY, J. H. Studies in the physiology of the cambium. New Phytol. **29**: 56–73, 96–140, 316–374. 1930.
159. RABER, O. Water utilization by trees. U. S. Dept. Agr. Misc. Pub. 257. 1937.
160. REED, JOHN F. Root and shoot growth of shortleaf and loblolly pines in relation to certain environmental conditions. Duke Univ. School For. Bull. **4**: 1–52. 1939.
161. R. G. S. The Kansas City seminar. Bull Amer. Met. Soc. **16**: 135–136. 1935.
162. SALISBURY, E. J. The plant and its water supply. Jour. Roy. Hort. Soc. **62**: 425–442. 1937.
163. SALISBURY, E. J., AND JANE, F. W. Charcoals from Maiden Castle and their significance in relation to the vegetation and climatic conditions in prehistoric times. Jour. Ecol. **28**: 310–325. 1940.
164. SALTER, R. M. Some soil factors affecting tree growth. Science **91**: 391–398. 1940.
165. SAMPSON, A. W. Principles and methods of tree-ring analysis. [A review of book by W. S. Glock.] Jour. For. **36**: 80–82. 1938.
166. ————. Rainfall and tree growth in the Great Basin. [A review of book by E. Antevs.] Jour. For. **36**: 1249–1251. 1938.
167. ————. Plant indicators—concept and status. Bot. Rev. **5**: 155–206. 1939.
168. ————. The dendrochronology enigma. Jour. For. **38**: 966–968. 1940.
169. SCHULMAN, E. Douglass on climatic cycles and tree growth. Bull. Amer. Met. Soc. **19**: 204–211. 1938.
170. ————. Nineteen centuries of rainfall history in the Southwest. Bull. Amer. Met. Soc. **19**: 211–215. 1938.
171. ————. Classification of false annual rings in west Texas pines. Tree-Ring Bull. **6**: 11–13. 1939.
172. ————, AND BALDWIN, G. C. The chronology in OL. 12, a dissected ponderosa. Tree-Ring Bull. **6**: 13–16. 1939.
173. SCHUMACHER. F. X., AND DAY, B. B. The influence of precipitation upon the width of annual rings of certain timber trees. Ecol. Mono. **9**: 387–429. 1939.
174. SCHUMACHER, F. X., AND MEYERS, H. A. Effect of climate on timber-growth fluctuations. Jour. Agr. Res. **54**: 79–107. 1937.
175. SERRANO, L. L. Moisture contents of clay loam at the time of wilting of rice plants of different ages. Philippine Agr. **27** (7): 530–547. 1938.
176. SHREVE, FORREST. A montane rain-forest. Carnegie Inst. Wash. Pub. 199. 1914.
177. ————. The growth record in trees. Carnegie Inst. Wash. Pub. 350: 89–116. 1924.
178. ————. Rainfall, runoff, and soil moisture under desert conditions. Annals Assn. Amer. Geogrs. **24**: 131–156. 1934.
179. SHREVE, FORREST, MALLERY, T. D., AND TURNAGE, W. V. Desert investigations. Carnegie Inst. Wash. Yearbook 37: 223–227. 1938.
180. SILLIMAN. Longevity of trees. Amer. Jour. Sci. **22**: 379. 1832.
181. SNEDECOR, GEORGE W. Statistical methods. *In*: Methods in plant physiology, by W. E. Loomis and C. A. Shull: 383–416. 1937.
182. SPERRY, O. E. The rate of growth of the ponderosa pine in Estes Park, Colorado. Bull. Torrey Bot. Club **61**: 19–34. 1934.
183. ————. A study of the growth, transpiration, and distribution of the conifers of Rocky Mountain National Park. Bull. Torrey Bot. Club **63**: 75–103. 1936.
184. SPOEHR, H. A. Form, forces, and function in plants. Coop. Res. Carnegie Inst. Wash. Pub. 501: 435–462. 1938.
185. STALLINGS, JR., W. S. Dating prehistoric ruins by tree-rings. Lab. Anthr., Gen. Ser., Bull. **8**: 1–20. 1939. Sante Fe, New Mexico.

186. STETSON, HARLAN T. The sun and the atmosphere. Smith. Inst. Annual Rept. 1938, Pub. 3491: 149–174. 1939.
187. STREIFF, A. Study of climatic cycles of interest to hydraulic engineers. Civil Eng. **5**: 488. 1935.
188. THOENNES, GREGORY. [Note in Science, Suppl. **93** (2407): 9. 1941.]
189. THOREAU, HENRY D. A week on the Concord and Merrimack Rivers. Edited by Odell Shepard. 1921.
190. TONZIG, SERGIO. La distribuzione e la funzionalita dell'acqua nella cellula vegetale (Distribution and functioning of water in plant cells). Nuovo Gior. Bot. Ital. **45** (4): 419–557. 1938 (1939). [From Biol. Abs.]
191. TOUMEY, J. W., AND KORSTIAN, C. F. Foundations of silviculture. 1937.
192. TURBERVILLE, H. W., AND HOUGH, A. F. Errors in age counts of suppressed trees. Jour. For. **37**: 417–418. 1939.
193. TURESSON, G. Die Pflanzenart als Klimatindikator. Kungl. Fysiog. Sällsk. Lund. Forhandl. **2**: 1–35. 1932. [From Biol. Abs.]
194. TURNER, L. M. Catastrophies and pure stands of southern shortleaf pine. Ecology **16**: 213–215. 1935.
195. VEIHMEYER, F. J., AND HENDRICKSON, A. H. Soil-moisture conditions in relation to plant growth. Plant Phys. **2**: 71–82. 1927.
196. WALTHER, H. Die Periodizität von Trocken- und Regenjahren in Deutsch-Südwestafrika auf Grund von Jahresringmessungen an Bäumen. Ber. Deut. Bot. Ges. **54** (10): 608–620. 1936.
197. WEAKLY, H. E. Tree-rings as a record of precipitation in western Nebraska. Tree-Ring Bull. **6**: 18–19. 1940.
198. WEAVER, J. E. The ecological relations of roots. Carnegie Inst. Wash. Pub. 286. 1929.
199. WIGHT, W. Radial growth of the xylem and the starch reserves of *Pinus sylvestris;* a preliminary survey. New Phytol. **32**: 77–96. 1933.
200. WILDE, S. A. The relation of soils and forest vegetation of the lake states region. Ecology **14**: 94–105. 1933.
201. WILLEY, G. R. Notes on Central Georgia dendrochronology. Tree-Ring Bull. **4**: 6–8. 1937.
202. WILLIAMSON AND CLARK. Variability of annual rainfall in India. Geog. Rev. **21**: 675–676. 1931.
203. YOCUM, W. W. Root development of young delicious apple trees as affected by soils and by cultural treatments. Neb. Agr. Exp. Sta. Res. Bull. 95: 1–55. 1937.

RECENT DEVELOPMENTS IN PLANT DISEASES IN THE UNITED STATES

NEIL E. STEVENS
University of Illinois
AND RUSSELL B. STEVENS
Birmingham-Southern College

INTRODUCTION

The present summary of information regarding certain diseases in the United States through the season of 1940 is in large part a continuation of one published earlier in this journal (23) which contained information up to and including 1935. As in the earlier paper, the choice of diseases to be included has been determined by the present interest of the disease due to its recent appearance in this country or to marked fluctuations in extent or severity, and by the completeness or reliability of available information regarding its incidence. Miss Jessie I. Wood, who collaborated in the preparation of the earlier paper, has aided in this one with numerous valuable suggestions.

Much of the material here summarized is, of course, taken from the Plant Disease Reporter. For economy and convenience, references to this publication are made merely by volume and page, italicized, in parentheses inserted in the text.

THE DUTCH ELM DISEASE

The history of the Dutch Elm Disease (caused by *Graphium ulmi*) in the United States is so well known that only the merest outline will be given. Certainly no attempt will be made to review the literature of this disease, which included up to May, 1937, 678 titles (8).

The disease was first discovered on a few trees in Cleveland and Cincinnati, Ohio, in 1930. Naturally, there was much speculation as to how a fungus recognized in Europe since 1919 as the cause of a serious disease of an important shade tree could have entered the country. No satisfactory explanation, not even a really interesting theory to account for this fact, was offered until after the discovery in June, 1933, of a much more severe infection in New Jersey. In September of that year, however, Gravatt and Fowler (9) announced that burl elm logs from France had been found at

the ports of Baltimore and Norfolk bearing not only the fungus causing the disease, but two species of *Scolytus* beetles which had been found by European investigators to be carriers of the fungus. Importation of these logs and their shipment to various points in the United States for making veneer were found to have been going on for a number of years.

Since 1933 there has been carried on by the Federal Department of Agriculture and various state agencies an extensive campaign for the control of the disease with three general objectives—to determine as completely as possible the distribution of the disease and its known insect carriers, to reduce by sanitation both the amount of infective material and the number of insect carriers by reducing suitable breeding places, and to eradicate so far as possible the infected trees.

The results to January, 1941, are summarized by E. G. Brewer (3) in an article in American Forests from which the accompanying map (Fig. 1) was taken, as follows:

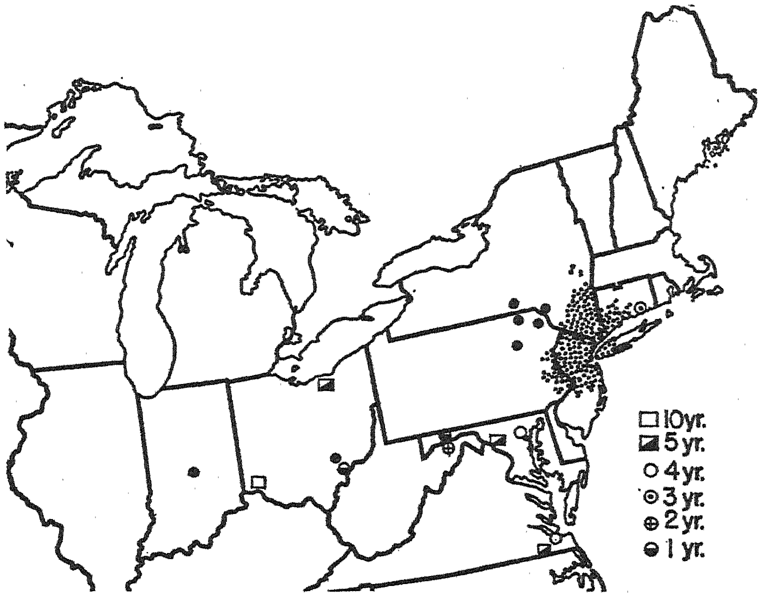


FIG. 1. Dutch elm disease infection areas, 1930–1940. Stippled portion represents the 1940 infection area in New York harbor vicinity. Black dots indicate outlying infections in which disease was found during 1940. Other signs indicate outlying infections that did not recur in 1940 and, in each case, the period of freedom from disease.

"There is one isolated point of infection, Cincinnati, Ohio, at which no diseased trees have been located for 10 years. There are three isolated points of infection, Brunswick, Maryland; Cleveland, Ohio, and Portsmouth, Virginia, where no diseased trees have been found for five years. At two isolated points, Baltimore, Maryland, and Norfolk, Virginia, there has been no recurrence of the disease for four years, and there are other points where no diseased trees have been found for one, two or three years."

In a number of the isolated localities indicated by black dots on the map, infection was still present in 1940, and the major region of infection—centering around New York City—covered in that year some 10,000 square miles. In all, up to the end of 1940, 60,975 diseased trees had been found in the United States.

WASTING DISEASE OF EELGRASS

The economic importance of eelgrass, *Zostera marina* L., was so little recognized that it was not until its sudden and almost complete disappearance from Atlantic coastal waters in 1931–32 that most botanists came to realize its direct, as well as indirect, usefulness. Indeed, it was the starving of brant and Canada geese on their winter feeding grounds off the coast of North Carolina and elsewhere that first called general attention to the fact that their usual food, eelgrass, was almost completely lacking. In England it was observed that swans were deserting the swaneries for the same reason.

Prior to this outbreak, *Zostera marina* occurred abundantly in tidal waters on mud flats from Beaufort, North Carolina, to the Gulf of St. Lawrence. Reproducing largely by root stalks, the plants formed dense masses which were frequently acres, or even miles, in extent. Especially at low tide, these masses were hard to penetrate and served as an excellent shelter for various forms of marine life, as well as a source of irritation to numerous boatmen. In favorable situations, such as the Bay of Fundy, the leaves were regularly gathered and sold as an insulating material.

Although in the late spring of 1931 at least a large part of the stands were normal, by midsummer of 1932 this common, native plant had largely disappeared. Naturally, so striking a phenomenon resulted in numerous local reports, much speculation and some research. It is impossible to enumerate all the scattered local records, many of which are cited by Renn (18), but it is agreed that by midsummer eelgrass had largely disappeared throughout its entire range on the Atlantic seaboard of North America and that

the patches which survived were almost wholly near the mouths of rivers or in other regions of reduced salinity. The phenomenon was also observed in 1931–32 on the Atlantic coast of France and Spain; the channel coast of France, England and Holland and the coast of southern Ireland (16). In 1933 it appeared on the coast of Norway and Sweden and in Danish waters.

No comparable change was observed in Pacific waters. In the reports summarized by Cottam (7) in 1939 it is indicated that *Zostera marina* has remained unaffected in American Pacific coastal waters. We have found no specific records of the recent abundance of this plant in Asiatic waters, but a paper published in 1935 (12) certainly suggests it was abundant in Japanese waters at that time.

In the course of studies of the wasting disease, at least two different organisms have been found growing as parasites on diseased eelgrass on the Atlantic coasts of both America and Europe, a species of *Labyrinthula* (17 & 20) and a fungus described as *Ophiobolus halimus* Mounce and Diehl (14 & 26). Although the ability of these organisms to parasitize *Zostera marina* has not been questioned, the opinion appears to be fairly general among investigators that the sudden and destructive disease may well have been associated with some weakening of the host plant from environmental causes. The situation is further complicated by the report of the *Labyrinthula* in eelgrass (29) from the Pacific Coast of North America.

Perhaps second in interest only to the question of the causes of eelgrass scarcity is the question of whether similar phenomena have appeared in the past. Cottam, who has given some time to the study of available records, concludes (5) that "there have been past periods of eelgrass scarcity, probably however, not to be compared in intensity or completeness with the present catastrophe." Only two actual printed records, however, were found—in 1894 in Massachusetts and 1913 in France. Since 1933 all available reports indicate a gradual, though by no means regular or continuous return of eelgrass on the Atlantic coast of North America (19, 13, 6) (*24: 116–118, 394* and *25: 46–52*).

Early in August, 1941, Renn informed the writers that he had confirmed the presence of the *Labyrinthula* in *Zostera marina* from a number of points along the coast of California, and recently (Nov. 4, 1941) Cottam writes regarding the condition of

eelgrass that there apparently has been a marked diminution in certain areas, primarily from northern California to southern British Columbia.

X-DISEASE OF PEACH

The X-disease of peach, at times referred to as "yellow-red virosis," was first described by Stoddard (24) in 1938. At that time it was known chiefly from Connecticut, with one infection noted in the Hudson Valley region of New York. Since the publication of this first paper there have been additional reports of infection on peach from New York (*22: 268*) (10) and from Massachusetts (*22: 334, 23: 341–42*). Early in the investigation of this disease, the common chokecherry (*P. virginiana*) was found to be a natural host, and the existence of the disease on this species alone has been established in Vermont (10), Illinois (*23: 328; 24: 340–341*), Wisconsin (*23: 386*), New Hampshire and Rhode Island; and is suspected in western Pennsylvania. The situation west of the Great Plains area is complicated somewhat by the difference in climatic conditions, and the presence there of what is considered by many a variety of the eastern chokecherry, *P. virginiana* var. *demissa,* by others a distinct species, *P. demissa.* There is a suspicion, however, of the occurrence of X-disease on peach in Colorado (10), Idaho (*23: 216*), Washington (*24: 388*), Utah (11) and California (25); and on the western chokeberry in Utah (10). (See Fig. 2.)

Thus far, attempted transmission by means other than budding or grafting have proved unsuccessful, suggesting that X-disease is a virus of somewhat the same nature as peach yellows, and the likelihood of natural transmission by insect vector. No insect has yet been proven responsible for this spread. The virus, as already indicated, occurs naturally on peach, nectarine and chokecherry; and produces visible symptoms when budded into the western sand cherry, *P. Besseyi* (*25: 361*). The suggestion is that the latter may be an additional wild host in the western portion of this country. The disease has not yet been found on the common wild cherry, *P. serotina,* and apparently cannot be artificially induced in this species.

Economically, X-disease is important to the orchard areas of Massachusetts, Connecticut and New York; and to the nurseries

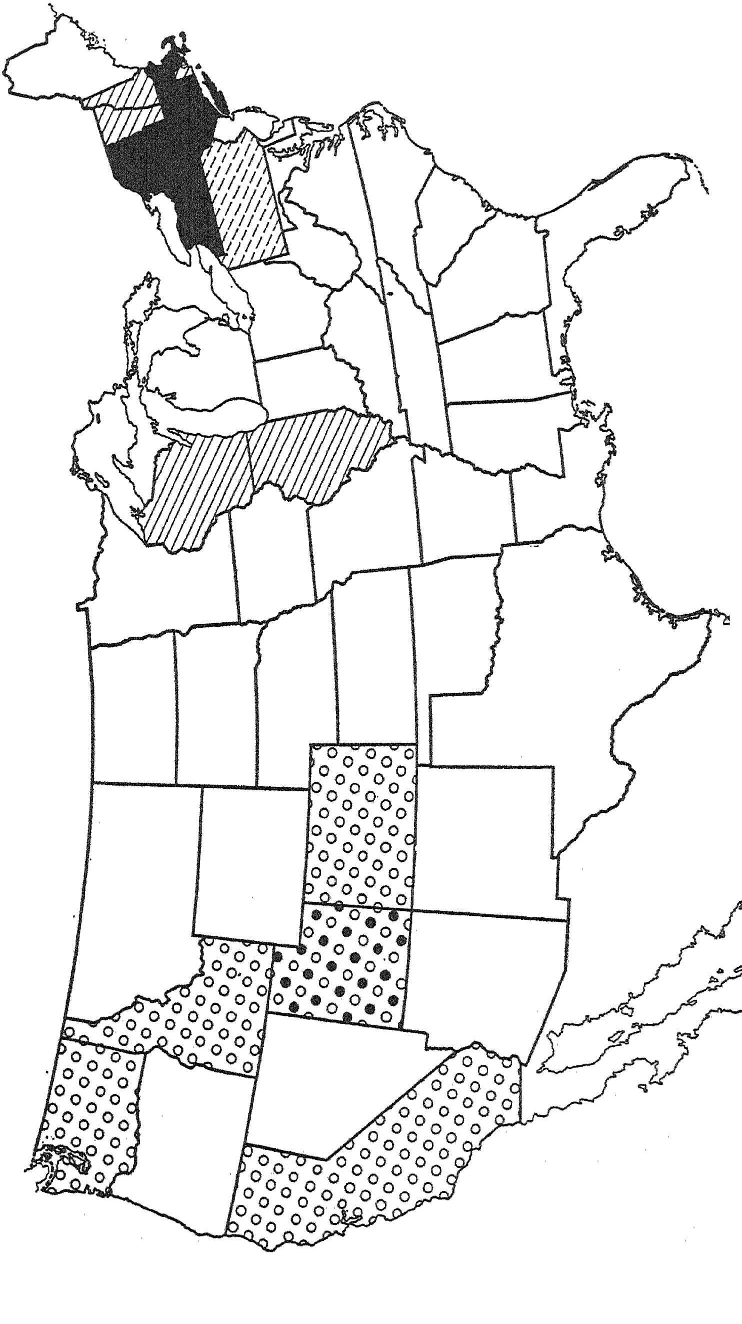


FIG. 2. X-disease of peach, 1940. Solid black indicates states in which X-disease is known on peach; unbroken shading, on chokecherry. White dots represent suspected occurrence on peach; broken shading, suspected occurrence on chokecherry; and black dots. suspected occurrence on western chokecherry.

of that region as well. Although diseased orchard trees seldom if ever die outright, they are soon rendered commercially worthless, and the disease has the ability to destroy the usefulness of an entire orchard in short order. In four orchards held under observation from 1933 to 1937, Stoddard (24) found that from 30% to 95% of the trees became diseased; while Hildebrand and Palmiter (10) cite a typical disease incidence of 65% three growing seasons after planting. Boyd (*23: 341–342*) considered it in 1939 more serious to the peach industry than any other one, or even two, diseases. Complete removal of the natural chokecherry host, thereby removing the source of infection near the orchard, seems to offer the best control measure at present. In the absence of such control, the disease appears capable of rapid spread and disastrous results.

The widespread, though somewhat discontinuous, occurrence of the disease on chokecherry brings up the question of its origin. Stoddard (24) records its presence as early as 1933 in Connecticut, and feels it must have been present for "some time previous"; Hildebrand and Palmiter (10) believe it occurred in the Hudson River Valley "possibly for as long as 4 years" prior to 1938. Since chokecherry affected with the virus will show the first season a yellowing of the leaves, often developing by late July into strikingly brilliant red and scarlet coloration, it seems unlikely that the disease could have gone unnoticed for any great length of time. The consensus would appear to be that it may possibly have been present to an inconspicuous degree for some time, but that it has only in the last decade developed to serious proportions.

During the summer of 1941 X disease was found on peach and chokecherry in Michigan (P.D.R. 25: 406) and its presence on peach in Colorado was confirmed (P.D.R. 25: 474).

DOWNY MILDEW OF TOBACCO

In the introductory sentences to the previous account (23) of tobacco downy mildew, caused by *Peronospora tabacina* Adam, the writers had no intention of raising again the once vexed question of earlier records of the fungus or the disease in the United States. They certainly did not intend to pass judgment as to the region from which the fungus may have reached the commercial tobacco areas of the eastern United States. If their statements may be so construed, they were not correctly written.

Wolf has taken a great interest in this problem, and the information regarding species of *Peronospora* on wild and cultivated *Nicotianae* collected in both North and South America, prior to 1921, was summarized by him in 1939 (*27*). As a result of his examination of available specimens and a study of the evidence, Wolf concluded that proof as to whether the tobacco downy mildew pathogen had been present in the United States prior to its discovery in Florida and Georgia in 1921, was still lacking. Wolf (*28*), in 1939, also reported the presence of downy mildew on tobacco in Brazil and said: "It must be concluded, therefore, that two species of *Peronospora* occur on *Nicotiana* in South America. The one, *P. nicotianae,* has long been present, but it is highly probable that *P. tabacina* was only recently introduced. Of course, the manner of its introduction into Brazil, just as into the United States, remains unknown." From the foregoing it is apparent that any reasonably reliable record of the history of downy mildew as a disease of cultivated tobacco in the United States begins with 1921.

During the years 1931–1935 tobacco downy mildew was present in varying degrees of severity in the tobacco regions of the United States. As shown by maps published earlier (*23*: figs. 1–4), the extreme limits of its distribution were west to Louisiana, 1931, and central Tennessee, 1933, north to southeastern Pennsylvania, 1932. The importance of the crop and the general distribution of the disease have resulted in much study of control measures.

As regards incidence and distribution during 1936–40 (Fig. 3), chief interest has naturally attached to its appearance in areas not previously affected. In 1936 the disease was first found in Kentucky in Todd County near the Tennessee line (*20: 180*). In 1937 the disease was very severe in the southern tobacco regions and for the first time destructive losses occurred in fields, as distinguished from seed beds or under shade. That year it not only reappeared in Kentucky, finally being found in 28 counties, but also was found in Indiana (*21: 246*) and the Connecticut Valley region of Connecticut and Massachusetts (*21: 218*). On June 7, 1938, downy mildew was found for the first time in Canada near the town of Essex in Essex County (*22: 289*). In 1939 it appeared in Miami County, Ohio (*23: 177*).

The disease reappeared in Massachusetts and Connecticut in 1938–39–40, reaching in 1938 Franklin County, Mass., the north-

ernmost section of the tobacco growing area. In the Connecticut Valley region of Connecticut and Massachusetts, as well as in some sections of Florida, much tobacco is grown under "tents" for cigar

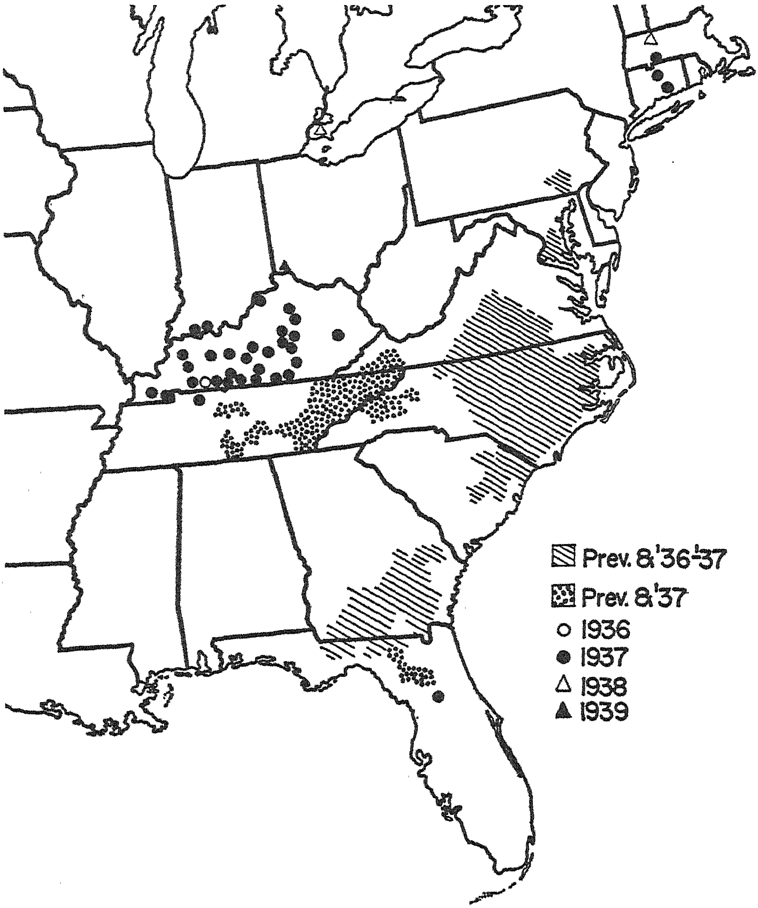


FIG. 3. Tobacco downy mildew, 1936–1939. Shaded portion indicates area of previous occurrence affected in both 1936 and 1937; stippled portion area of previous occurrence affected in 1937 only. Other signs represent location of counties in which the disease was first found in year indicated.

wrappers. Under these conditions the disease in 1938 caused serious damage to large plants. The fungus produced spores and spread from plant to plant as late as July 10. Losses in these tents

were particularly noticeable in the Connecticut Valley in 1939 and 1940, estimated by the Connecticut Experiment Station at Windsor at $100,000 in both years; and were reported from Florida in 1939 (*23: 383*).

BACTERIAL WILT OF CORN

The commercial importance of bacterial wilt of sweet corn (caused by *Phytomonas stewarti*) has been very greatly reduced by the spectacular success of Golden Cross Bantam in largely replacing the open pollinated varieties. Sufficient acreages of susceptible varieties are still planted, particularly for the early market, to make it possible to trace the fluctuations in range of this disease with some degree of accuracy.

It will be remembered that following exceptional and in some places extremely heavy losses from this disease in sweet corn north of the Ohio and Potomac rivers in 1932 and 1933, including even parts of Maine and Michigan, there was a very marked reduction in the amount of the disease in 1934 and 1935. During the five years 1936–1940, considerable fluctuations in the severity of the disease in this area were noted. The available information has been published regularly in the Plant Disease Reporter and was summarized with citations in Volume *25: 152–157*.

Losses from this disease were negligible in this area in 1936 and 1940. In 1936, indeed, susceptible varieties made good stands and produced a fair crop of marketable ears even at Arlington Farm, Virginia (*21: 104–105*). The 1937 reports showed a decided increase. In southern Connecticut there was an evident increase after three years with practically no wilt. In Nassau County on Long Island and in Rockland and Westchester Counties in southeastern New York State, the disease was widespread and was very severe in some plantings and on some varieties. In New Jersey there was much more than in 1936.

In 1938 there was some reduction, as compared with 1937, in losses due to bacterial wilt in the states mentioned above as showing heavy losses. The disease was severe only during the early part of the season. On the other hand, there was a decided increase in Indiana and Illinois. In Illinois bacterial wilt and *Diplodia* stalk rot combined caused severe injury to field corn over a wide area—losses of 40% to 50% in yield were reported in some farm fields in some hybrids in the corn performance tests. In 1939 bacterial wilt was reported as present but less severe than in 1938 for southeast-

ern New York and was somewhat more severe than in 1938 in Pennsylvania, New Jersey, Ohio, Indiana and Illinois.

The fluctuations in the range of this disease during the years 1936–1940 in the northeastern states follow closely the changes in winter temperature in the same area. Whether any causal relation exists, or whatever its nature, the correlation has proved sufficiently close to make possible accurate and serviceable forecasts of the probable incidence of the disease based on the mean temperatures of December, January and February (Fig. 4).

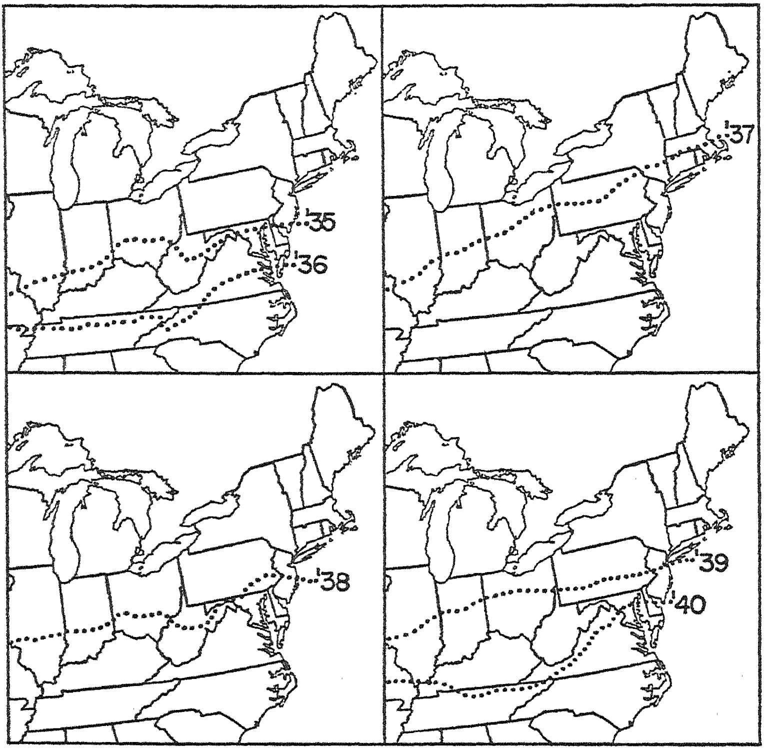


FIG. 4. Approximate northern boundaries (and also approximate northern limit of commercial losses from bacterial wilt of sweet corn) of areas with winter temperatures corresponding to an index (sum of mean temperatures of Dec.–Feb.) of 100 or more, for the years 1935–1940.

BUNT OF WHEAT

The fluctuations in the amount of bunt of wheat (caused by *Tilletia* spp.) in certain western states are shown in Figs. 5 and 6.

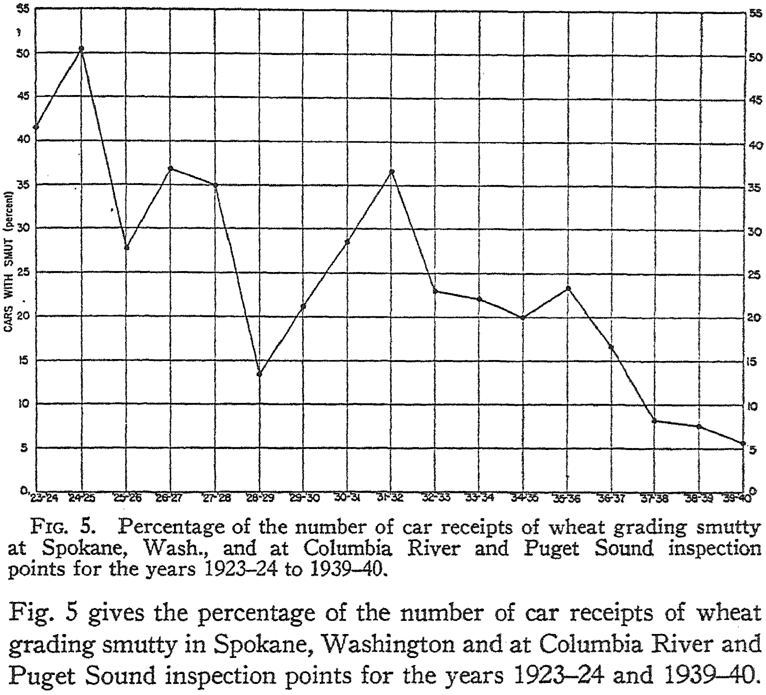


FIG. 5. Percentage of the number of car receipts of wheat grading smutty at Spokane, Wash., and at Columbia River and Puget Sound inspection points for the years 1923–24 to 1939–40.

Fig. 5 gives the percentage of the number of car receipts of wheat grading smutty in Spokane, Washington and at Columbia River and Puget Sound inspection points for the years 1923–24 and 1939–40. Fig. 6 gives the same information for Utah inspection points. Parts

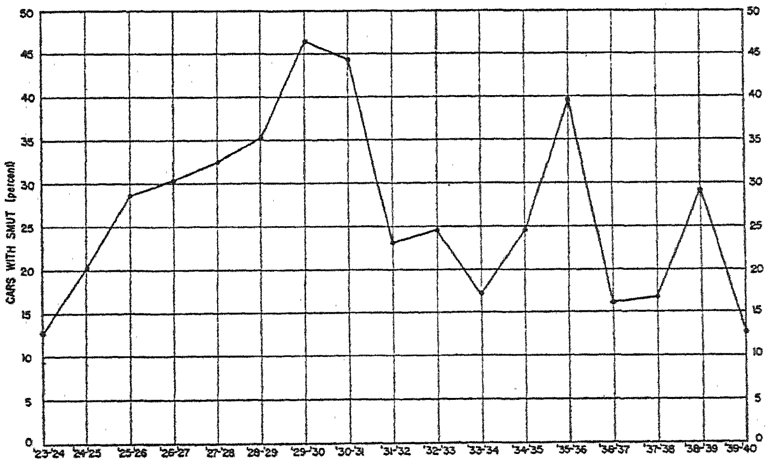


FIG. 6. Percentage of the number of car receipts of wheat grading smutty at Utah inspection points for the years 1923–24 to 1939–40.

of one of these figures were reproduced in the "Grain Inspectors' Letter" for October, 1939, and in the "Extension Pathologist" for October, 1937.

The completed figures were, however, sent to us by B. W. Whitlock, in charge, Pacific Coast Headquarters, Federal Grain Supervision, who also gave us permission for their publication. The accuracy of these reports of Federal grain inspection and their usefulness as a source of plant disease information has already been commented upon, and the correlation between the amount of bunt in the field and the smuttiness of threshed grain, has been specifically noted (*19: 71–93; Sup. 79: 1–5*, 1931).

CERCOSPORELLA FOOT ROT OF WHEAT

From 1928 to 1939 losses due to *Cercosporella* foot rot of winter wheat (caused by *C. herpotrichoides*) in Washington, Oregon and Idaho, were carefully compiled by Roderick Sprague and his associates. The estimated losses in bushels for the various years are shown in Fig. 7. The figures for 1928 to 1935 have been published (21, 22).

Those for 1936 to 1939 were furnished by Sprague in a recent letter and that for 1940 was given us by Dr. Wayne Bever, who was in the region at the time and has long been familiar with conditions there. All of these figures are, of course, based on observations by numerous field workers.

Sprague finds (22) that weather conditions have a profound effect on the development and severity of *Cercosporella* foot rot and that the conditions that tend to favor its development are: sufficient soil moisture to insure immediate germination of wheat in the fall; long growing season in the fall, accompanied by abundant rain, resulting in excessive fall growth; warm, wet weather in February and March with cold, wet weather in April, which retards grain growth; and continuation of wet weather into May, followed abruptly by a dry period.

As will be seen from the graph (Fig. 7), 1934 was the worst year within the period under review and Sprague notes that the preceding winter was almost frost free and that other conditions favored the development of the disease.

STEM RUST OF WHEAT

Stem rust of wheat has been the subject of intensive study in this country for many years. Information regarding its history

is, accordingly, unusually detailed and accurate. Surely in few, if any, important diseases are the fluctuations in losses larger or more drastic.

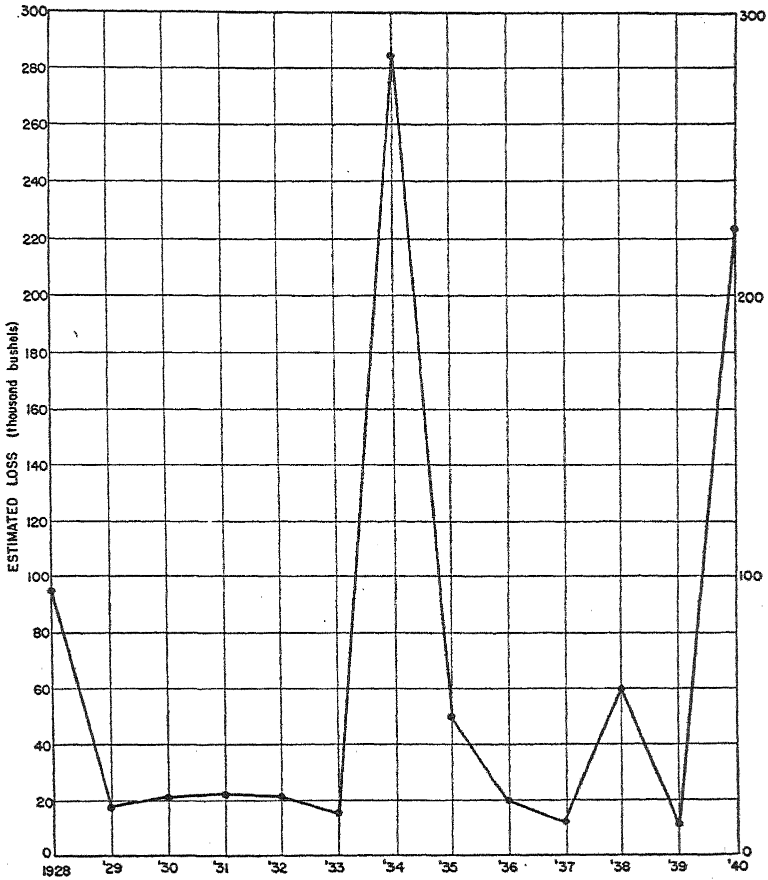


FIG. 7. Estimated losses due to *Cercosporella* foot rot of winter wheat in Washington, Oregon and Idaho, 1928–1940.

Following the exceedingly severe outbreak of this disease in 1935, losses were at a minimum in nearly all wheat growing areas in 1936. In 1937 they were again heavy, the estimated loss for the reporting area being 9.3%, which has been exceeded (*23: 295*) only twice since the Plant Disease Survey was established in 1917. The dis-

ease was particularly severe in the spring wheat region. The loss in southeastern North Dakota, for example, was placed by Stakman at 60% (*21: 292*). Frequent reports from the field, published in various numbers of the Plant Disease Reporter, as the season progressed, indicated that very much heavier losses might have occurred but for the fact that large areas were planted to rust resistant varieties of wheat, notably the variety Thatcher (*21: 274* and *305*). In a careful review of the developments in Kansas, including weather conditions and varietal resistance, Johnston, Melchers and Miller say (*Sup. 107: 83–94.* 1938) that in some localities rust damage was greater than that recorded for the severe epidemic of 1935.

As early as July, 1938, Stakman (*22: 337*) reported that "In general stem rust in 1938 will not be nearly so destructive as in 1935 and 1937," and this was confirmed by later reports and estimates. The unusual severity of stem rust in Idaho and Washington during 1938 was emphasized by Bever (*22: 346* and *372*).

Stakman and Hamilton summarize the situation in 1938 as follows:

> "Both stem rust and leaf rust were epidemic on wheat in 1938. Stem rust was unusually heavy in many of the principal grain-growing areas of the Southern and Central States, becoming particularly destructive in central Texas, in some fields of eastern Oklahoma, on soft wheat in northeastern Kansas, and in sections of eastern Nebraska. In much of northern Missouri soft wheat also was heavily rusted, although the loss was not so great as it was in 1937. The epidemic was severe in the spring wheat areas on susceptible varieties, reaching its greatest intensity in considerable areas of North Dakota. In other parts of the spring wheat States rust development was checked by the predominance of the resistant Thatcher wheat, as in Minnesota and in certain sections of northeastern North Dakota, or by hot dry weather, as in parts of North Dakota and South Dakota." (*Sup. 117: 1*).

In contrast to conditions in 1937 and 1938 stem rust of wheat was, in general, relatively unimportant in the Plains region in 1939 and 1940. It was, however, very severe in California in 1940, a loss of 60–70% in the Imperial Valley having been reported by Sunenson (*24: 239–240*).

LEAF RUST OF WHEAT

That losses from wheat leaf rust (caused by *Puccinia rubigo-vera tritici*) were unusually high in 1938 is apparent from the fact that the average crop loss estimate reported to the Plant Disease Survey was 9.6% as compared with the previous highest average estimate

of 5% in 1935 and with an average estimated loss of 1.5% for the decade 1925–34 (Fig. 8).

Among important wheat producing states which indicated heavy losses due to this disease were Iowa 28%, Oklahoma 27%, Nebraska, Missouri and Tennessee each 20%, and Illinois 15%.

Numerous early reports from Texas (*22: 103–240* & *242*), Oklahoma (*22: 133–135*), Kansas (*22: 143*) and elsewhere indicate that leaf rust appeared unusually early throughout the southern Plains region (*22: 151–158*).

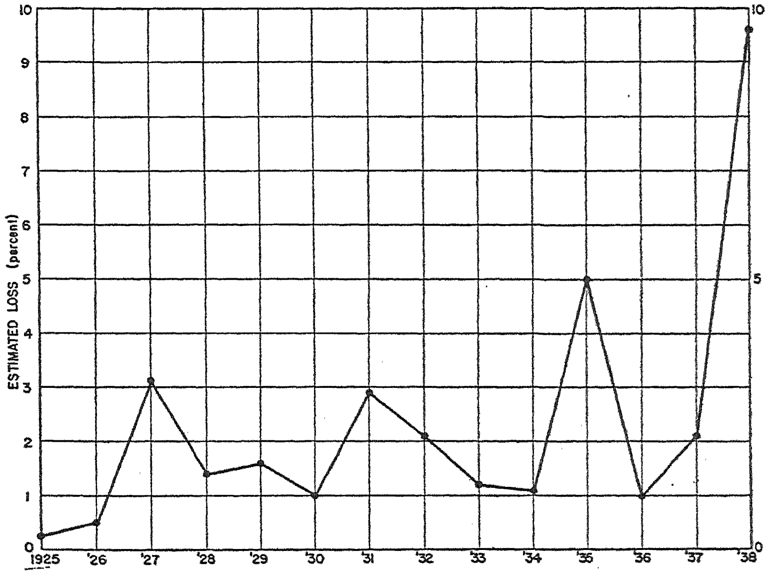


Fig. 8. Estimated losses from leaf rust of wheat in the United States (reporting area), 1925–1938.

The 1938 outbreak of leaf rust in Oklahoma was made the subject of a special report by K. Starr Chester (*Sup. 112.* 1939). His estimate of losses due to this disease cited above was based on actual field observation during special surveys, and he quotes veteran wheat growers and wheat specialists as agreeing "that the 1938 outbreak was the earliest and most extensive which they can remember." He called attention to the probability that here in the past, as in other states, damage really caused by leaf rust has at times been attributed to stem rust.

In discussing the relation between the 1938 outbreak and weather conditions he says:

"The first three months of 1938 were all abnormally warm, the departures from normal being + 3.9°, + 5.4°, and + 7.3° for January, February, and March, respectively. Precipitation was approximately normal for January, but in February a 50-year record high began a period of excessive rainfall which was to carry through till harvest, the accumulated precipitation by June 30 being 23.28 inches as compared with 16.86 inches for a normal year."

Similar conclusions were reached by Melchers who says (*Sup. 116:66*):

"Weather and crop conditions in Kansas have seldom been so favorable for the development of a leaf rust epidemic as they were in 1938."

Naturally following such heavy losses the development of the disease during the early part of 1939 was watched with much interest. All reporters agree, however, that while there was abundant inoculum in some places, the weather which was cool and dry was not favorable to the development of the disease, and losses were relatively light in the southwest.

BEAN RUST

Bean rust, caused by *Uromyces phaseoli typica,* was unusually abundant in 1936. This is indicated by reports from a number of states, though the severity of the losses naturally varied greatly in the different localities. The accuracy of these reports is attested by the fact that they came from competent field observers who were able on the basis of their own experience to compare conditions in 1936 with other recent years.

Zaumeyer, Wade & Mullin (*22: 43*) refer to 1936 as the first year in which bean rust had been noted to any extent since 1928 in Colorado. According to these observers it was even more prevalent in that state in 1937, but in neither year was there serious commercial damage.

In Florida, on the other hand, the estimated loss from rust on snap beans was set at 22%. Townsend and Tisdale say specifically (*20: 133*):

"Bean (*Phaseolus vulgaris* L.) rust caused by *Uromyces phaseoli typica* Arth. has been a serious disease on the late summer and fall crops of Kentucky Wonder beans in the northern part of the State since 1923, when this variety was first grown extensively in that area. In the southern portion of the State bush beans, principally the Bountiful variety, are grown extensively and mostly during the fall and winter months. Rust occurs in most localities of this portion of the State every year, but as a rule, causes no damage and often is never observed by the growers. Such slight infections had been con-

sidered to be due in part to the fact that the Bountiful has been considered to be only slightly susceptible to rust. However, this theory has proved erroneous during the 1935–36 season.

"Rust was first observed in November of this season in the east coast section between West Palm Beach and Fort Lauderdale and became more severe on successive plantings until early in March. Losses from the disease in that section have been uniformly heavy and in many cases the crop was a total failure. Several thousand acres were not picked at all and the yield on most of the remaining acreage was low, averaging about 25 per cent of a normal yield for the season."

The disease was also reported as locally severe in Mississippi (*20: 173*). For southeastern Massachusetts Boyd reported that he "had never before seen it so abundant in bush beans." Boyd's observations relate particularly to Bristol and Plymouth counties, but those of Guba on Nantucket Island indicate that conditions were comparable there (*20: 303*). Regarding conditions in New Jersey, Haenseler says (*20:292*):

"Our past records going back to 1913 show only traces or occasional infested fields annually but this fall the disease is of state-wide distribution and the cause of serious defoliation in many fields."

Cook reported it as severe on the eastern shore of Virginia (*20: 327*).

From Washington state Huber reports (*20: 332*) that while the disease has been present in Washington for several years, it had caused little injury to the crop. Rust was very severe in 1936.

"At harvest time, it was estimated that out of the 400 acres of Blue Lake and Kentucky Wonder varieties, principally the former, the rust disease caused approximately 40 per cent reduction in yield in Whatcom County during 1936."

In 1938 this disease was locally severe also in southern Florida (*22: 61*), Long Island, New York (*22: 331*) and eastern Virginia (*22: 332*).

POTATO LATE BLIGHT

Some idea of the severity of potato late blight (caused by *Phytophthora infestans*) in 1938 may be gained from the fact that the estimated reduction in yield for the reporting area was given as 12.8%. This figure is over three times the average for the ten years 1920–29 and over four times the average for 1925–34, and, of course, it includes many states where the disease was not important (Fig. 9). The estimated loss for the northeastern states was 27.6% with Massachusetts indicating a 35% and New York a 45% loss.

On the map, Fig. 10, are indicated the states which reported heavier loss from late blight in 1938 than other recent years either in their estimates or verbal reports to the Plant Disease Survey

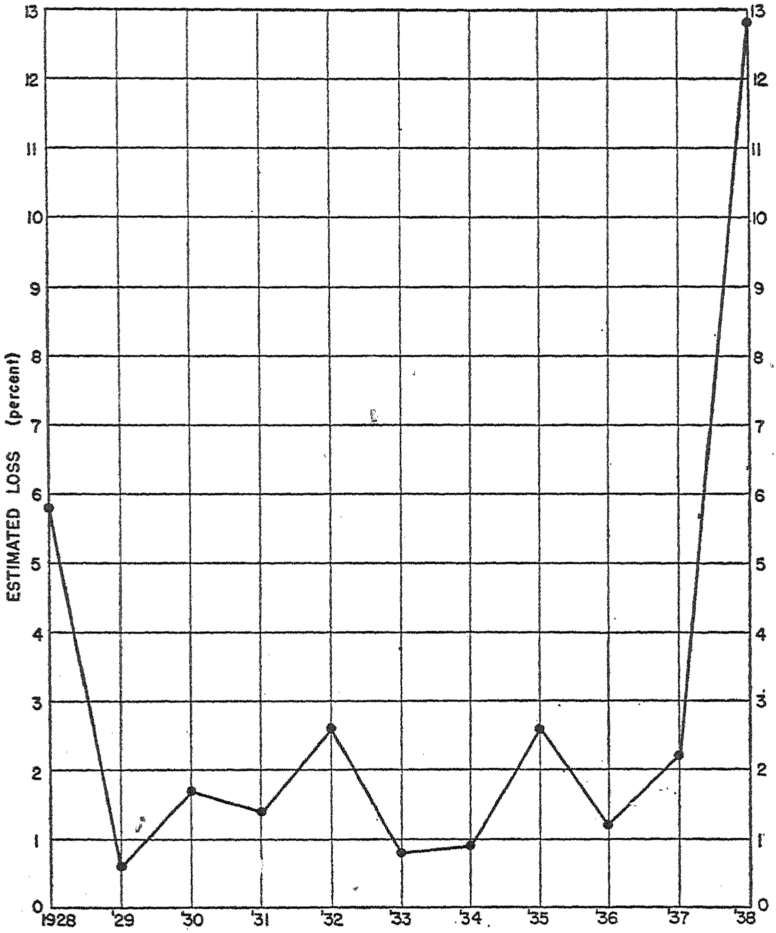


FIG. 9. Estimated losses from potato late blight in the United States (reporting area), 1928–1938.

(mostly in *22: 412–422*) and in "Sectional Notes" published in the American Potato Journal, Vol. 15: 211, 234, 236, 268, 289, 292, 295. These reports often say that 1938 is the "worst year in ten years" although that from New Jersey says it is the worst in twenty years.

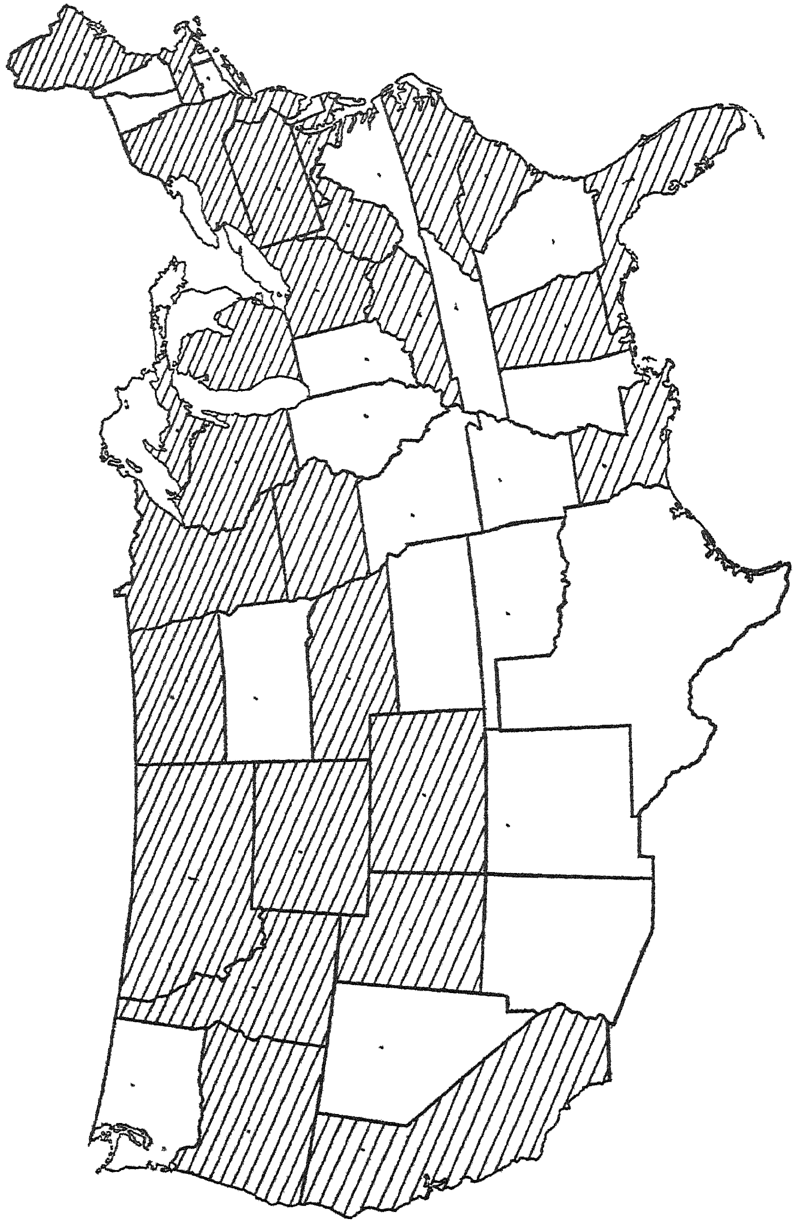
In 1940 late blight was of minor importance in the New England States (*24: 452–460*), and less than in 1938–39 in Delaware, Maryland, West Virginia, Florida. Losses were above average, how-

FIG. 10. Potato late blight. Shaded portion indicates those states which reported the disease as more important in 1938 than in other recent years (see text).

ever, in New York, New Jersey, the Upper Peninsula of Michigan, and Wisconsin. In Louisiana (*24: 253*) the disease was observed for the first time since 1928, and in one locality caused the most widespread damage ever observed in the state.

BACTERIAL RING ROT OF POTATO

Early in 1940 Leach, Goss and Dykstra, acting as a Committee for the Potato Association of America, published in the Plant Dis-



ease Reporter (*24: 2–6*) and in the American Potato Journal (17: 81–88) a report on the distribution of ring rot of potatoes (caused by *Phytomonas sepedonica*) in the United States. Their map, based on a survey by several interested agencies, showed the disease to be definitely reported in 27 states (Fig. 11). These writers emphasize the point that negative reports do not necessarily mean that the disease does not occur in a state, for in many cases a very thorough survey had not been made. It was thus apparent that this disease was widely distributed though the evidence available to the committee indicated that it was only locally abundant in most states. During 1940 the presence of the disease was established in Arkansas (*24: 234*), Virginia (*24: 252*) and Washington (*24: 115* and *440*).

While ring rot is apparently regarded by most of those who have studied it to be of relatively recent introduction, it may well be that it is already too late to get adequate information regarding the history of its spread. At present the following information is available. Bonde in 1937 (2) described a "bacterial soft rot" from Aroostook County, Maine, where it had appeared annually since 1932, the year in which he first observed it. Within a few months of the publication of Bonde's paper, the disease was recognized in Florida (*21: 271–273*) where there was attributed to it an estimated loss of 5%.

What is believed by many workers to be the same disease was described from Quebec in 1931 (1).

It remained for Burkholder (4) to identify the American disease with the Bakterienringfäule described in Germany in 1913 and apparently recognized there earlier. Burkholder's conclusions as to the identity of the disease seems to have been generally accepted in the United States. The Canadian workers (15), as late as 1938, appeared to be still reserving judgment.

LITERATURE CITED

Much of the material here summarized is taken from the Plant Disease Reporter. References to this publication are made merely by volume and page (italicized) in parentheses inserted in the text.

1. Baribeau, B. A wilt disease of potato. Canad. Pl. Dis. Sur. Rep. **11**: 49. 1931.
2. Bonde, Reiner. A bacterial wilt and soft rot of the potato in Maine. Phytopathology **27**: 106–108. 1937.
3. Brewer, E. G. The fight for the elms. Am. For. **47**: 22–25. 1941.

4. BURKHOLDER, W. H. The occurrence in the United States of the tub. ring rot and wilt of the potato (*Phytomonas sepedonica*) (Spieckermann a. Kotthoff) Bergey *et al.* Am. Potato Jour. 15: 243–24 1938. [Also in earlier papers therein cited.]
5. COTTAM, CLARENCE. Past periods of eelgrass scarcity. Rhodora 36: 261–264. 1934.
6. ————. Status of eelgrass (*Zostera marina*) on the North Atlant coast. Feb. 1938. Wildlife Res. & Man. Lft. BS–110. April 1938
7. ————. The eelgrass situation on the American Pacific Coast. Rhodora 41: 257–260. 1939.
8. GOSS, MARIE C., AND C. S. MOSES. A bibliography of the Dutch Elm Disease. Unnumbered Mim. Pub. U. S. Bur. Pl. Ind. 61 pp. 1937.
9. GRAVATT, G. F., AND M. E. FOWLER. Log interceptions at Norfolk in relation to the entry of tree diseases. Pl. Dis. Rep. 17: 129–131. 1933.
10. HILDEBRAND, E. M., AND D. H. PALMITER. Yellow-red virosis (X-disease) of peach and chokecherry. Pl. Dis. Rep. 22: 394–396. 1938.
11. HUTCHINS, LEE M. X-disease of peach and chokecherry. Unnumbered Mim. Pub. U. S. Dept. Agr., Sept. 25, 1939.
12. ISHIDA, S. Changes in sea water in relation to the abundance of the *Zostera* plant [Translated title]. Botany and Zoology, Tokyo, Japan 3: 612–616. March 1935.
13. LYNCH, JOHN J., AND CLARENCE COTTAM. Status of eelgrass (*Zostera marina*) on the North Atlantic Coast, January 1937. Wildlife Res. & Man. Lft. BS–94. July 1937.
14. MOUNCE, I., AND W. DIEHL. A new *Ophiobolus* on eelgrass. Canad. Jour. Res. 11: 242. 1934.
15. RACICOT, H. N., D. B. O. SAVILLE, AND I. L. CONNERS. Bacterial wilt and rot of potatoes. Some suggestions for its detection, verification and control. Am. Potato Jour. 15: 312–318. 1938.
16. RENOUF, L. P. W. *Zostera* disease on the coast of County Cork. I. F. S. Nature 133: 912. 1934.
17. RENN, C. E. A mycetozoan parasite of *Zostera marina*. Proc. VI Int. Bot. Cong. II. 203. 1935.
18. ————. Wasting disease of *Zostera marina*. Bio. Bull. 70: 148–158. 1936.
19. ————. The eelgrass situation along the middle Atlantic Coast. Ecology 18: 323–325. 1937.
20. SPARROW, F. K. Discussion. Proc. VI Int. Bot. Congr. I: 282. 1936.
21. SPRAGUE, RODERICK. Influence of climatological factors in the development of *Cercosporella* foot rot of winter wheat. U. S. Dept. Agr. Circ. 451: 1–39. 1937.
22. ————. *Cercosporella* foot rot of winter grains in the Pacific Northwest. Unnumbered Mim. Pub. Div. Cereal Crops & Dis., U. S. Bur. Pl. Ind., Dec. 8, 1939.
23. STEVENS, N. E., AND J. I. WOOD. Recent fluctuations in plant diseases in the United States. Bot. Rev. 3: 277–306. 1937.
24. STODDARD, E. M. The "X-disease" of peach. Conn. Agr. Exp. Sta. Circ. 122: 54–60. 1938.
25. THOMAS, H. EARL, T. E. RAWLINS AND K. G. PARKER. A transmissible leaf-casting yellows of peach. Phytopathology 30: 322–328. 1940.
26. TUTIN, T. G. *Zostera marina* and a fungal parasite. Proc. VI Int. Bot. Cong. II: 203–204. 1935.
27. WOLF, F. A. Status of investigations of tobacco downy mildew. Phytopathology 29: 194–200. 1939.
28. ————. Downy mildew of tobacco in Brazil. Phytopathology 29: 291. 1939.
29. YOUNG, E. L., III. *Labyrinthula* on Pacific Coast eelgrass. Canad. Jour. Res. C. 16: 115–117. 1938.